Front. Biol. 2013, 8(2): 160–174
DOI 10.1007/s11515-012-1202-6

REVIEW

The balancing act of AKT in T cells

Mary Catherine RENEER, Francesc MARTI (✉)

Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky College of Medicine, Lexington, KY 40536, USA

Abstract **The serine/threonine-specific protein kinase AKT is gaining recognition as a major crossroad in numerous cellular signaling pathways through its ability to regulate cell differentiation, proliferation, survival and metabolism. This review focuses on the recent advances in AKT signaling and downstream events in T cells, emphasizing its contrasting role in conventional and regulatory (Treg) T cell populations. Activation of AKT has been known for many years to be critical in the development and function of conventional T cells. However, it has just recently been uncovered that AKT exerts an inhibitory effect on Treg generation and suppressor function. These studies have placed AKT at the nexus of Treg development and function, thus opening novel avenues for therapeutic manipulation.**

Keywords AKT, Tregs, T cell signaling, T cell differentiation

Introduction and historical background

To sense the changes in their surrounding microenvironment, cells utilize a variety of specialized cell membrane receptors that interact with molecules/ligands in the local milieu. The ligand-receptor interaction initiates a chain of intracellular biochemical reactions –pathways— that relay, amplify and distribute those initial signal inputs into the cell. Cross talk among multiple, simultaneously active pathways, together with positive and negative feedback activation loops, produce a complex signaling network that is tightly coordinated and integrated to summon the adequate cell response. This response is elaborated in the cell nucleus and mediated by the action of transcription factors through the regulation of gene expression and protein synthesis.

The serine/threonine-specific protein kinase AKT, also known as protein kinase B (PKB) is gaining recognition as a major crossroad in numerous cellular signaling pathways involved in the regulation of vital cell functions such as differentiation, proliferation, glucose metabolism and survival. As such, dysfunctional AKT is observed in many pathological conditions, including cancer (Staal, 1987), autoimmune disease (Rondinone et al., 1999), neurodegenerative conditions (Shimoke and Chiba, 2001) and muscle hypotrophy (Turinsky and Damrau-Abney, 1998), making it an attractive pharmacological target in current drug discovery programs.

The AKT research can be traced back to 1977 when Staal et al. (1977) reported the isolation and characterization of AKT8, a murine virus that caused a high incidence of spontaneous lymphoma. The viral oncogene was termed v-AKT and, later on, in 1991, the cellular homolog was identified as c-AKT (Bellacosa et al., 1991). Additionally, two other groups looking for members of the protein kinase C (PKC) and protein kinase A (PKA) superfamilies, identified a gene that encodes a serine/threonine protein kinase which they named PKB (Coffer and Woodgett, 1991; Jones et al., 1991). Both c-AKT and PKB were found to be the same protein. Further studies demonstrated the critical involvement of AKT in growth factor signaling and oncogenesis. In 1995, it was reported that the lipid kinase phosphatidylinositol 3-kinase (PI3K) is directly upstream of AKT (Burgering and Coffer, 1995; Franke et al., 1995) which established the linkage of the PI3K-AKT pathway to metabolic functions such as protein and lipid synthesis, carbohydrate metabolism and transcription. As the field evolved, a new prospective on the role of AKT in growth, survival and metabolism became established and, with it, the foundation for the current compelling interest in the molecular regulation of AKT activity.

Received December 13, 2011; accepted January 4, 2012
Correspondence: Francesc MARTI
E-mail: fmart3@uky.edu

Overview of AKT activation

Protein structure of AKT

AKT belongs to the cAMP-dependent protein kinase A/protein kinase G/protein kinase C (AGC) superfamily of protein kinases. Each AGC member exhibits similar enzymatic function and structural homology within their catalytic domains (Song et al., 2005). Three different AKT genes have been identified in mammalian cells: AKT1/PKBα, AKT2/PKBβ and AKT3/PKBγ (Jones et al., 1991; Cheng et al., 1992; Altomare et al., 1995; Konishi et al., 1995; Brodbeck et al., 1999; Nakatani et al., 1999). Although AKTs are expressed in all eukaryotes, the specific isoforms and level of expression varies depending on the tissue type. AKT1 is the dominant isoform in the majority of tissues and is critical during embryonic development (Hanada et al., 2004; Yang et al., 2004; Dummler and Hemmings, 2007), AKT2 is expressed mostly in insulin-responsive tissues and has non-redundant functions in glucose homeostasis (Altomare et al., 1995) and AKT3 is restricted to testis and brain and is required for postnatal brain development (Konishi et al., 1995; Brodbeck et al., 1999; Nakatani et al., 1999).

The three AKT isoforms share a high degree of amino acid identity (~85%) and a common structural pattern that includes an N-terminal pleckstrin homology (PH) domain, a central kinase domain and a C-terminal regulatory domain with a hydrophobic motif (HM) (Fig. 1). The PH domain is a protein module of approximately 120 amino acids best known for its ability to bind to cell membrane phosphoinositides. The AKT PH domain preferentially recognizes phosphatidylinositol-3,4,5-triphosphate (PtdIns (3,4,5)P_3) and, with threefold lower affinity, PtdIns (3,4)P_2 (Rong et al., 2001; Thomas et al., 2002). Unlike PtdIns (4,5)P_2 and PtdIns-4P, which are constitutively produced in cells, PtdIns (3,4,5)P_3 and PtdIns (3,4)P_2 are detected only in stimulated cells (Carpenter and Cantley, 1996) thus providing an additional mechanism to regulate the recruitment of proteins such as AKT to the membrane. Next to the PH, the kinase domain shares a high similarity with other AGC kinases and contains the catalytic function of AKT that exhibits a preference to phosphorylate the canonical consensus sequence RXRXX(S/T) (Obata et al., 2000). It contains the regulatory Threonine 308 (T308) which phosphorylation is required for AKT activation. AKT possesses a C-terminal regulatory domain characteristic of the AGC kinase family with an FXX(F/Y)(S/T)(Y/F) hydrophobic motif. This sequence contains Serine 473 (S473), the second regulatory residue which phosphorylation is necessary for full activation of the kinase. Together these regions make up a protein of approximately 56kDa (Alessi and Cohen, 1998).

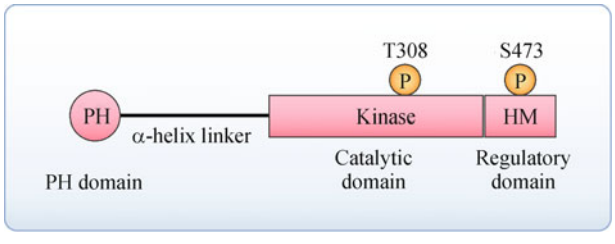

Figure 1 Structure of AKT. Depicted is the structure of AKT1. AKT consists of three domains: the PH domain, the kinase domain and the C-terminal regulatory domain with a hydrophobic motif (HM). The kinase domain contains T308, one of two residues that need to be phosphorylated in order for AKT to become active. The second residue, S473, is located in the hydrophobic motif of AKT.

Activation of AKT

Activation of AKT is a multi-step process that requires the receptor-ligand dependent activation of PI3K. PI3K is the lipid regulator that phosphorylates PtdIns (4,5)P_2 and, with lower efficiency, PtdIns 4P, at the 3′-OH position of the inositol ring to produce PtdIns (3,4,5)P_3 and PtdIns (3,4)P_2, respectively (Fig. 2). In the initial step of its sequential activation, AKT translocates to the cell membrane through the PH-mediated binding to PtdIns (3,4,5)P_3. This binding generates a conformational change in AKT that exposes the two regulatory phosphorylation sites, T308 and S473. The fact that PH domains of Phosphoinositide-Dependent Kinase-1 (PDK1) and AKT share their high affinity for PtdIns (3,4,5)P_3 favors their physical close proximity and enhances the efficiency of the PDK1-mediated phosphorylation of T308 in AKT. Phosphorylation of T308 only partially activates AKT and it is not yet clear whether it is a necessary prior event for phosphorylation of S473 (Yung et al., 2011). To reach full activation though, AKT must be phosphorylated at both T308 and S473. The identity of the kinase responsible for phosphorylation of S473 remains still controversial and several candidates have been reported, including PDK1/2 and the mammalian target of rapamycin (mTOR) associated within the mTOR complex-2 (mTORC2) (Balendran et al., 1999; Hill et al., 2001; Sarbassov et al., 2005; Peifer and Alessi, 2008; Alessi et al., 2009). The same kinase mTOR that is upstream of AKT as mTORC2, assembles within mTORC1, which is a direct AKT substrate. Phosphorylation by AKT of another member of both mTORC1 and mTORC2 results in opposite effects on the AKT pathway: while phospho-TSC1-2 sustains a positive feedback loop between mTORC2 and AKT (Huang and Manning, 2009), in some instances (for example, in the insulin receptor pathway, it mediates the inhibitory feedback loop of PI3K-AKT-mTORC1 axis (Harrington et al., 2005). This complex interplay between mTOR and AKT, mediated by mTORC1 and mTORC2 coordinates and diverts the AKT signaling to specific sets of downstream pathways that will define the functional response of the cell.

Overactivation of AKT can lead to the loss of proliferative and cell survival control that occurs in a wide array of solid

tumors and hemopoetic malignancies. To maintain proper physiologic function, AKT activity needs to be exquisitely balanced. Cells counterregulate AKT function via distinct mechanisms, including the degradation of PtdIns $(3,4,5)P_3$ by inositol phosphatases. Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) antagonizes PI3K activity and dephosphorylates the 3′-OH position, generating PtdIns $(4,5)P_2$ (Stambolic et al., 1998). The SH2-containing inositol polyphosphate 5-phosphatase (SHIP1) exhibits 5′-OH phosphatase activity to produce PtdIns $(3,4)P_2$ (Cantley and Neel, 1999; Roget et al., 2008). Both events lead to the formation of inositol lipids whose binding affinity for AKT is significantly lowered. The PH domain-only protein PH-like domain family A member 3 (PHLDA3) competes with AKT for binding to both PtdIns $(3,4,5)P_3$, and PtdIns $(3,4)P_2$, and the overexpression of PHLDA3 may block the localization of AKT to the membrane (Kawase et al., 2009). In addition, AKT can be directly targeted by the action of phosphatases such as protein phosphatase 2 (PP2A) and the PH domain leucine-rich repeat protein phosphatase (PHLPP). While PP2A preferentially dephosphorylates AKT at T308 and, under certain conditions, also targets phospho-S473 (Andjelković et al., 1996; Liao and Hung, 2004; Pim et al., 2005; Rocher et al., 2007; Padmanabhan et al., 2009), PHLPP dephosphorylates AKT specifically at S473 (Fig. 2) (Gao et al., 2005; Brognard et al., 2007; Mendoza and Blenis, 2007).

Pharmacological targeting of the AKT pathway

The physiologic relevance of AKT and the potential involvement of the AKT pathway in numerous pathological conditions have attracted major interest in AKT as a

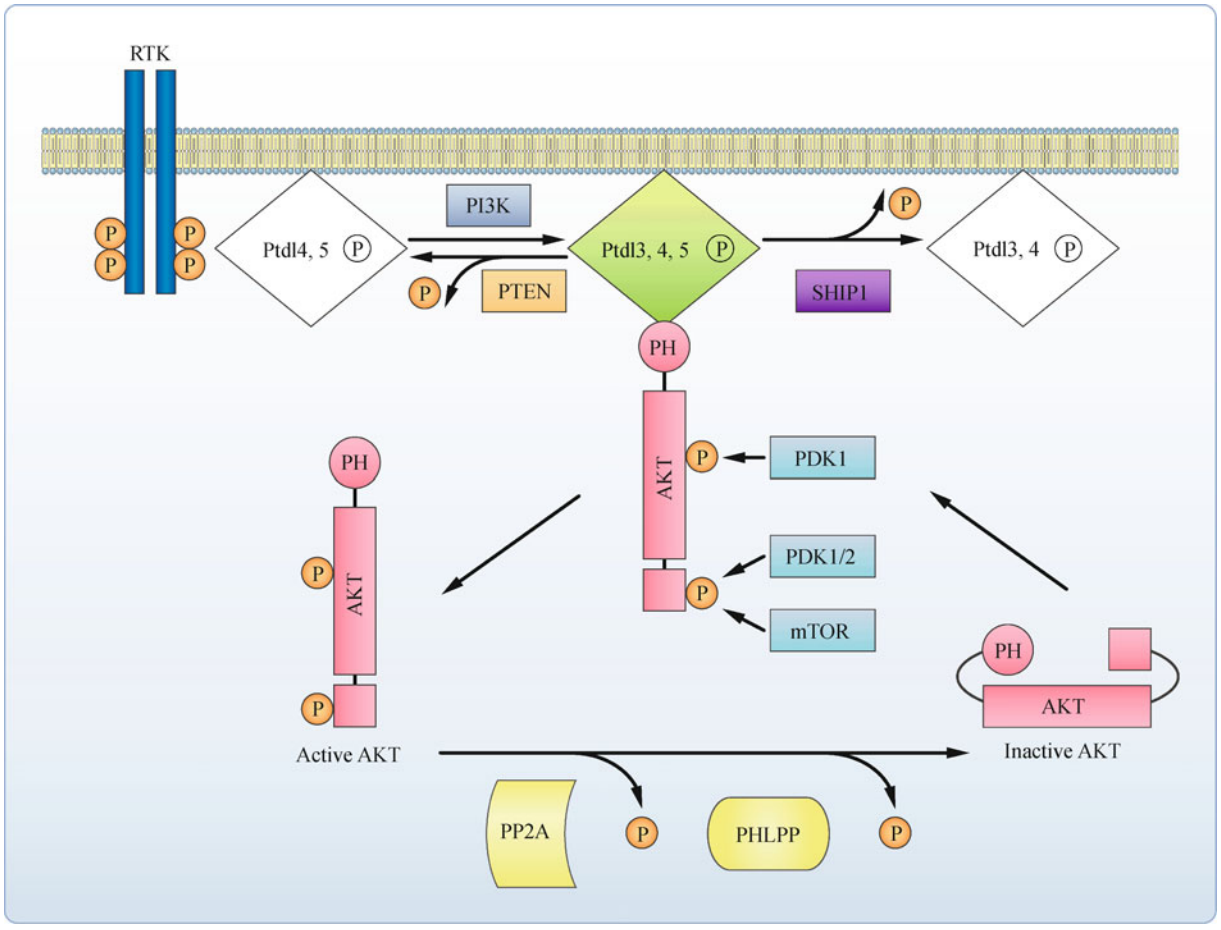

Figure 2 Regulation of AKT Activation. Upon ligand binding to receptor tyrosine kinase (RTK), PI3K is recruited to the membrane where it phosphorylates its substrate PtdIns $(4,5)P_2$ leading to the formation of PtdIns $(3,4,5)P_3$. PtdIns $(3,4,5)P_3$ will induce AKT translocation to the membrane by binding to its PH domain and causing a conformational change that allows T308 and possibly S473 to become accessible to PDK1 and mTOR for phosphorylation. Once phosphorylated, full activation of AKT results in the regulation of multiple cellular events related to cell proliferation, growth and survival. AKT activity is negatively regulated by the action of PtdIns phosphatases PTEN and SHIP which activity reduces the availability of the AKT-activating PtdIns $(3,4,5)P_3$. Lastly, PP2A and PHLPP directly target AKT by dephosphorylating phospho-T308 and phospho-S473, respectively and reverting AKT to an inactive conformation.

pharmacological target. One such drug is the fungal metabolite rapamycin, which is currently being used as an immunosuppressant to prevent rejection following organ transplantation. Rapamycin and its analogs rapalogs…, affect the AKT pathway by directly binding to the FKBP12 binding protein, which interacts preferentially with mTORC1 and blocks its downstream signaling activity. In some instances, inhibiting mTORC1 may result in pernicious effects by breaking the S6K-dependent inhibitory feedback that restrains the PI3K-induced AKT activation, i.e. eliciting the opposite intended outcome (O'Reilly et al., 2006). Consequently, alternative therapeutic strategies to rapamycin have been proposed by combining the inhibition of mTOR function and AKT activation. In this context, the strong structural similarities between the catalytic domains of mTOR and the p110 subunits of PI3K (Maira et al., 2008) facilitates the dual inhibitory activity of new chemical compounds, distinct from rapalogs, that are currently being tested in clinical trials (*clinicaltrial.gov* ID number NCT00620594). The most common PI3K-specific inhibitors, the synthetic compound Ly294002 (Vlahos et al., 1994) and the fungal metabolite wortmannin (Wiesinger et al., 1974), have been standard reagents in the experimental characterization of PI3K signaling, but their therapeutic use has been disregarded (Marone et al., 2008) and the attention has been shifted to a new generation of PI3K inhibitors that target the different isoforms of the regulatory p110 subunit. The potential therapeutic benefit of PtdIns analogs, has also been postulated. Nevertheless, despite their capacity to disrupt AKT activation, they may also generate specificity problems with respect to other PH domain-containing proteins. In contrast, the direct inhibition of AKT is considered a very attractive but challenging therapeutic option, either alone or in combination with other forms of therapy. Recently, several ATP-competitive inhibitors and novel allosteric inhibitors (PH domain-dependent small molecules) with selectivity for individual AKT isoforms are being actively characterized, some of which are in early phase of clinical trials or in clinical development (Hoffman et al., 1996; Hirai et al., 2010; Markman et al., 2010; Pal et al., 2010; Reid et al., 2011).

Active AKT profoundly affects cellular processes by phosphorylating numerous substrates. Consensus motif analyses reveal thousands of potential substrates for the enzymatic activity of AKT which can cause positive or negative regulation of the substrate. However, no more than 50 AKT substrates and the consequences that the phosphorylation by AKT may induce on their function, localization or protein stability have been characterized to date (Manning and Cantley, 2007). In this review we will focus on the recent advances in AKT signaling and downstream events in T cell lymphocytes, emphasizing its contrasting role in conventional and regulatory T cells (Tregs). For an extensive review of downstream signaling in other cell types, readers are referred to other excellent reviews on the subject (Kandel and Hay, 1999; Song et al., 2005).

The role of AKT in conventional T lymphocytes

The immune system is composed of two distinct yet complementary subdivisions: innate immunity and adaptive immunity. The innate arm generates the first line of defense against an infection or insult and reacts to pathogens rapidly but non-specifically, usually in response to pathogen-associated molecular patterns (PAMPs) expressed on infectious agents. The adaptive response, on the other hand, requires the antigen-specific recognition of pathogens and can take several weeks to develop. However, it generates a long-term protective memory that enables a rapid response upon a second encounter with the antigen-bearing pathogen. Two types of adaptive immune response can be differentiated: humoral, which is mediated by B cell produced antibodies, and cell-mediated, which involves the generation and activation of antigen specific T cells. Two major subsets of peripheral mature T cells are identified by the mutually exclusive expression of CD8 and CD4 surface molecules. Most $CD8^+$ T cells display cytotoxic effector function once activated. Normally, $CD8^+$ T cells recognize and lyse cells expressing the pathogenic antigen associated with MHC class I molecules. $CD4^+$ T cells, on the other hand, recognize foreign antigens in the context of class II self MHC, which enables them to respond only to professional Antigen Presenting Cells. Once activated, most effector $CD4^+$ T cells become helper T cells (T_H cells) that provide essential additional signals to activate and direct other cells to respond aggressively to an infectious agent. A minor population of $CD4^+$ T cells known as Tregs specializes in suppressing the activity of other lymphocytes and immune cells, helping to control the immune response and preventing collateral damage due to overactive inflammation. The development, activation and survival of T cells are indeed a tightly regulated and complex processes in which AKT plays a pivotal role.

AKT in T cell development

The thymic microenvironment directs the development, education and selection of T cells through the sequential integration of multiple extracellular cues that will ultimately generate the mature functional pool of circulating T cells. The first step in T cell development is the migration of early thymic progenitors (ETPs) from bone marrow to thymus (Penit and Vasseur, 1989). Once in the thymus, ETPs transition through four stages as double negative (DN) T cells (DN1-4) before becoming double positive (DP) $CD4^+CD8^+$ T cells (Godfrey et al., 1993). The emergence of a mature T cell requires that the developing thymocyte pass through several pre-T cell receptor (pre-TCR)/TCR dependent selection events, the first of which takes place at the DN3 stage, followed by positive and negative selection during the DP stage (Juntilla and Koretzky, 2008). Only DN3 thymocytes that express the newly created pre-TCRβ chain will

survive, a process known as β-selection (Levelt et al., 1993a, b). During the subsequent DP stage, additional positive and negative selection occurs in which phenotypic allelic exclusion at the TCRα locus is regulated and the reactivity of mature TCR for self-peptide/MHC takes place (Starr et al., 2003; Werlen et al., 2003; Juntilla and Koretzky, 2008). Although widely studied, the complete picture of signaling events and molecular mechanisms that control thymocyte differentiation is not well characterized. Experimental evidence suggests that AKT is essential during the transition from DN to DP, β-selection, allelic exclusion and the subsequent proliferation that follows DP transition, but the identification of specific pathways in which AKT is involved is still the focus of very active research (Sasaki et al., 2000; Hagenbeek et al., 2004; Hinton et al., 2004; Patra et al., 2006; Juntilla et al., 2007; Mao et al., 2007). A better understanding of the molecular events that control thymocyte development and T cell maturation will likely shed light upon the etiology and onset of a plethora of T cell-related pathologies, including autoimmune diseases and malignant transformations.

AKT in peripheral $CD4^+$ effector T cell differentiation

Following thymic selection and education, thymocytes are released into peripheral circulation as mature $CD8^+$ T cells or $CD4^+$ T cells. $CD4^+$ T_H cells have been classified into T_H1, T_H2 and T_H17, among others, according to their cytokine expression signature. T_H1 cells participate in cell-mediated immunity and are critical for the control of intracellular pathogens such as viruses and certain bacteria through the production of interferon (IFN)-γ and tumor necrosis factor beta (TNF-β). T_H2 cells direct B cell activation and antibody production as well as basophilic and eosinophilic inflammation through the secretion of interleukin (IL)-4, IL-13 and IL-5. T_H17 cells are critical in protecting the surface of skin and intestine against extracellular bacteria through the production of IL-17. AKT signaling is involved in the peripheral differentiation of distinct effector T_H cell subsets. Arimura et al. (Arimura et al., 2004) found that the expression of a constitutively active form of AKT induced T_H1 differentiation in C57BL/6 mice; however, AKT promoted T_H2 differentiation in BALB/C mice. Conversely, Kane et al. (Kane et al., 2001) reported that, also in BALB/C mice, CD28-induced AKT upregulated the T_H1 cytokines IL-2 and IFN-γ but not T_H2 cytokines. These discrepancies were reconciled by the findings of Lee et al. (Lee et al., 2010) who were investigating mTORC2. These authors created mice with a conditional deletion of rictor on C57BL/6 background, an essential subunit in the mTORC2 complex, and reported that both T_H1 and T_H2, but not T_H17 differentiation, were impaired. Additionally, they found that complementation with constitutively active AKT rescued only T_H1 differentiation in mTOR deficient mice, whereas activated PKC-θ restored T_H2 cells, implying that mTOR-dependent AKT is playing a pivotal role in the development of T_H1 subset, but not T_H2. AKT has been also reported to be crucial for the expression of T_H17 cytokines in $CCR6^+$ human memory T cells (Wan et al., 2011).

AKT activation in conventional T cells

The plethora of stimuli and pathways that regulate AKT function underlines its crucial role in T cells. One of the first events was documented by Reif et al. (Reif et al., 1997), showing that addition of IL-2 to a human T cell line lead to stimulation of AKT in a PI3K-dependent fashion. Later on, Lafont et al. (Lafont et al., 2000) reported that TCR ligation, like IL-2, also promoted a sustained, PI3K-dependent activation of AKT that lasts several hours (Lafont et al., 2000). Co-signaling through CD28 receptor is required for optimal TCR-dependent naïve T cell activation, cytokine production, cell survival and proliferation (Salomon and Bluestone, 2001). Parry et al. (Parry et al., 1997) reported that upon CD28 co-stimulation, TCR-induced, PI3K-dependent AKT activation increased substantially in primary human T cells. Upon activation, AKT integrates and relays multiple pathways that dictate downstream events that define the outcome of the T cell function and fate. Phosphorylation of downstream substrates of AKT leads to activation or deactivation of a myriad of regulatory events in T cells. Among them, glycogen synthase kinase 3 (GSK-3) modulates the synthesis of glycogen in lymphocytes. GSK-3 is constitutively activated in the absence of growth factors and directly regulates the nuclear factor of activated T cells (NFAT), a family of transcription factors involved in cytokine gene induction (Beals et al., 1997). NFAT activity is controlled by a nuclear import/export cycle governed by phosphorylation/dephosphorylation (Rao et al., 1997). NFAT is directly phosphorylated by GSK-3, and the phosphorylation and inactivation of GSK-3 by AKT indirectly promotes NFAT-dependent synthesis of pro-inflammatory cytokines in T cells (Fig. 3). Similar to NFAT, the eukaryotic initiation factor 2 (eIF-2B) involved in the translational control of protein synthesis, is another substrate of GSK-3 that is rescued by AKT (Kleijn et al., 1998).

AKT is also functionally linked with the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Fig. 3), which is involved in T cell development, maturation and proliferation (Livolsi et al., 2001). Kane et al. (Kane et al., 1999) found that the catalytically active AKT is necessary for the degradation of the NF-κB inhibitor IκB, although it is still controversial whether AKT alone is sufficient to induce NF-κB (Ozes et al., 1999; Romashkova and Makarov, 1999) and whether IκB is a direct target of AKT (Madrid et al., 2000; Sizemore et al., 2002). Regardless of the precise mechanism, it is currently clear that the PI3K/AKT/NF-κB signaling axis is operative in activated T cells.

The forkhead family of transcription factors (FoxO) plays an important role in cell proliferation and differentiation and

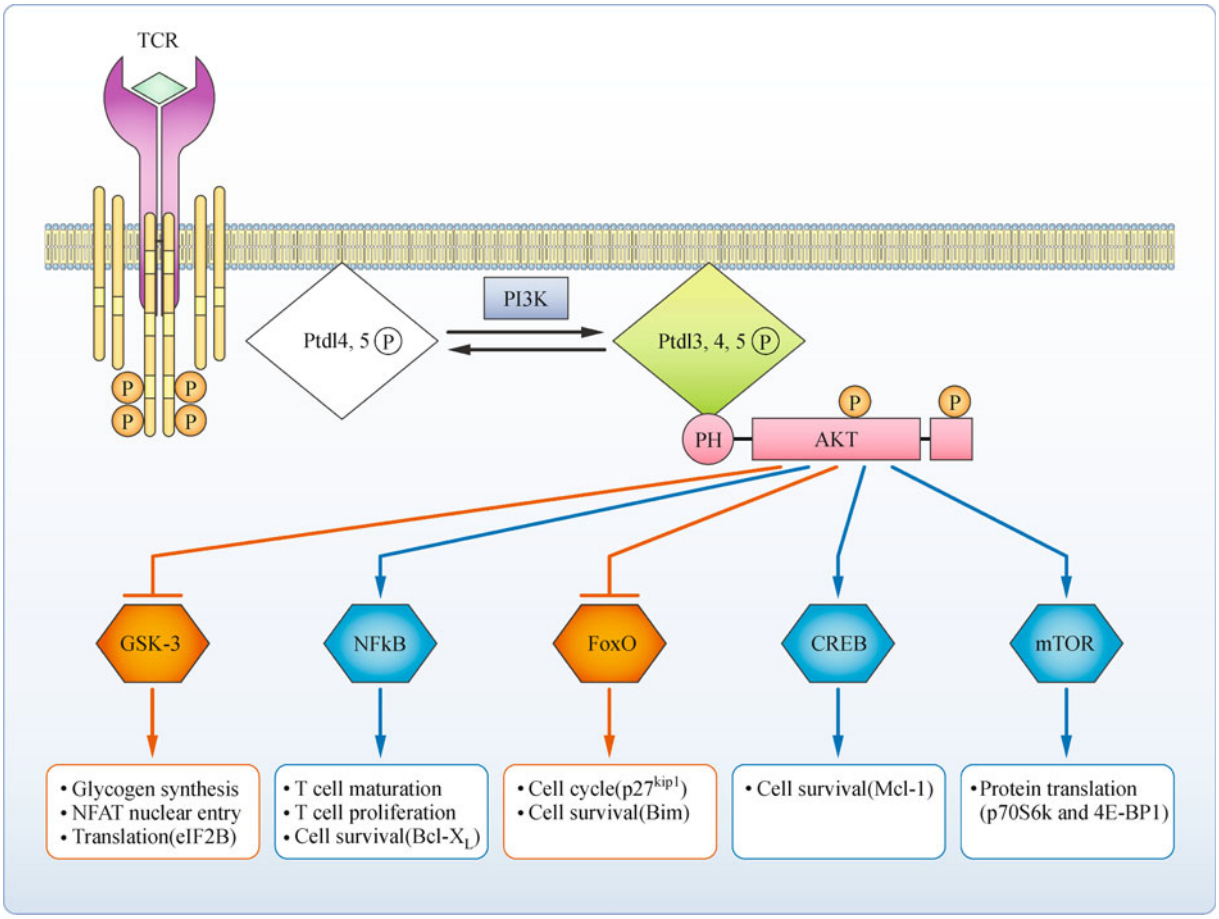

Figure 3 Metabolic functions of AKT in conventional T cells. Schematic outline of the downstream protein targets of AKT in conventional T cells, as discussed in the text.

is another major target of AKT kinase (Burgering and Kops, 2002). FoxO3 is involved in cell cycle regulation and survival of T cells by inducing the IL-2-dependent expression of the cell cycle inhibitor p27^{kip1} and the pro-apoptotic Bcl-2 family member Bim (Stahl et al., 2002) (Fig. 3). Like other transcription factors, FoxO activity is regulated by its nuclear localization and its phosphorylation by AKT precludes its nuclear accumulation and activity (Brunet et al., 1999; Burgering and Kops, 2002). In addition, AKT has been found to modulate the activity of another member of the Bcl-2 family, the pro-apoptotic BAD (Datta et al., 1997; del Peso et al., 1997). Upon phosphorylation, BAD dissociates from Bcl-2 and forms a very stable complex with 14-3-3 protein, which allows the free Bcl-2 to associate with and inhibit the pro-apoptotic effect of Bax. AKT has been found to regulate cell survival through other pathways, including the NF-κB-dependent upregulation of Bcl-X$_L$ (Chen et al., 2000; Khoshnan et al., 2000), the activation of the transcription factor cyclic adenosine 3′,5′-monophosphate (cAMP)-responsive element binding protein (CREB) (Du and Montminy, 1998) and the direct inactivation of human caspase-9 (Cardone et al., 1998).

With the caveat of the functional intricacies of the interaction between AKT and both of mTOR complexes, the observation that AKT can regulate mTOR activity opened the possibility that AKT can indirectly regulate protein translation (Fig. 3) by either enhancing ribosomal-dependent protein synthesis through the activation of p70S6 kinase (Jefferies et al., 1997; Redpath et al., 1996) and through the mTOR-dependent inactivation of the translation repressor 4E-BP1. In contrast with the fundamental role of AKT in conventional T cell function, results from recent studies are consistent with the negative effect of AKT signaling in the development and suppressor function of Tregs.

The role of AKT in regulatory T cell lymphocytes

Overview of Tregs

Overactivation of the immune system can lead to an excessive pathological inflammatory response and/or to autoimmune disease. Maintaining a balance between effective immune response and peripheral tolerance is achieved, in part, by Tregs (Annacker et al., 2001; Shevach, 2001) through direct

cell to cell contact, soluble factors, metabolic disruption or indirect effect mediated by dendritic cell (DC) suppression (Tang and Bluestone, 2008; Vignali et al., 2008; Shevach, 2009). First known as "suppressor" cells, for over a decade it remained unclear the true identity of these anti-inflammatory cells due to an inability to distinguish them from other conventional T cells. The turning point of Treg research came in 1998 with the link of the suppressor function with the cells that expressed high levels of the IL-2 receptor alpha chain (CD25) (Papiernik et al., 1998). Additionally, in 2003 the identification of the forkhead winged helix transcription factor (FoxP3) as a critical regulator of Treg development and function helped to establish a Treg phenotype (Brunkow et al., 2001; Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003). Deficiencies in FoxP3 underlie the lymphoproliferation and multiorgan autoimmunity of scurfy mutant mice and human patients with Immunodysregulation Polyendocrinopathy and Enteropathy, X-linked syndrome (IPEX) (Ziegler, 2006; Feuerer et al., 2009). IPEX usually affects males causing them to rarely live beyond infancy and suffering from severe enlargement of the secondary lymphoid organs, insulin-dependent diabetes, eczema, food allergies and concomitant infections (Workman et al., 2009). Despite their critical involvement in immune homeostasis, Tregs constitute only about 1%–5% of the circulating T cells in the human body. Two main subsets have been distinguished according to their origin: naturally occurring, thymically selected natural Tregs (nTregs) and peripherally induced Tregs (iTregs) differentiated from circulating conventional $CD4^+$ T cells. These two subsets are phenotypically indistinguishable in the expression of CD25, cytotoxic T lymphocyte antigen 4 (CTLA4), glucocorticoid-induced tumor necrosis factor receptor (GITR), and FoxP3 and lack the IL-7 receptor (CD127). Unfortunately, despite intensive research, there is still no surface or intracellular markers which clearly distinguish Tregs from conventional T cells in humans, although nTregs differ from iTregs in a variety of ways. While high affinity interactions with cognate self-peptide-MHC complexes and strong CD28 co-stimulation drive the development of nTreg, iTregs require weaker, suboptimal TCR stimulation and can be generated in the absence of CD28 co-stimulation. In addition, differentiation of iTregs, but not nTreg development, requires IL-2 and transforming growth factor-β (TGF-β) (Horwitz et al., 2008). nTregs have a TCR specificity for self-antigens in the thymus whereas the iTreg repertoire is more specific for tissue and foreign antigens (Bluestone and Abbas, 2003). This dichotomy may account also for their different regulatory activities, with nTregs preferentially involved in preserving self-tolerance and iTregs being more functionally active at inflammatory sites.

Careful attention must be paid to the differences between mice and human Tregs. While FoxP3 is considered the definitive marker for mouse Tregs, the expression of FoxP3 in human is not confined to Tregs, but it can be also induced in activated, conventional T cells. This observation added further confusion as to whether circulatory $FoxP3^+$ Tregs are thymic in origin and persist in the periphery or whether they were induced during antigen-specific stimulation (Akbar et al., 2007).

Peripherally induced Tregs

Key evidence for the relevance of peripheral generation of human Tregs came from the work of Vukmanovic-Stejic et al. The authors proposed that antigenic persistence induces a continuous generation of short-lived and prone to apoptosis Tregs from a population of rapidly dividing, highly differentiated memory $CD4^+$ T cells (Vukmanovic-Stejic et al., 2006;). Since the number and function of $CD4^+CD25^+FoxP3^+$ Tregs are maintained in humans even in senescence (Akbar et al., 2007), thymic nTregs cannot explain the lifelong existence of human Tregs, which supports the relevance of the peripheral development in the pool of circulating Tregs in adult humans. Challenging the standard paradigm of T cell lineage commitment, recent evidence emphasizes the functional and developmental plasticity (Heri et al., 2010) of T cells. In this context, iTregs can be generated from T_H1 (Hori et al., 2010), T_H2 (zheng et al., 2009), and T_H17 (Zhou et al., 2008) effector cells as well as from naive cells (Apostolou et al., 2002). In fact, $CD103^+$ DCs from the mesenteric lymph nodes can induce the differentiation of naïve $CD4^+$ T cells into iTregs in the mucosal environment of the gut, providing a mechanism by which functionally specialized gut-associated lymphoid tissue DCs can extend the repertoire of Treg cells (Coombes et al., 2007). All these findings are consistent with iTreg differentiation being tailored according to specific microenvironment requirements, thus ensuring the physiologic ability to control immune homeostasis when and where needed. In addition to their critical function in maintaining immune homeostasis after infection or inflammatory response, iTregs are also considered major contributors to the failure of immunosurveillance during cancer development, as the tumor microenvironment is especially favorable for the generation of iTregs (Josefowicz and Rudensky, 2009).

This high degree of developmental plasticity of iTregs makes them also functionally unstable. Xu et al. (Xu et al., 2007) and Yang et al. (Yang et al., 2008) found independently that a fraction of peripheral $FoxP3^+$ T cells lost FoxP3 expression and produced IL-17 upon *in vitro* stimulation in the presence of IL-6. Also, when highly purified $FoxP3^+$ cells were transferred into $RAG^{-/-}$ mice, approximately half of the cells lost their FoxP3 expression and produced IFN-γ, IL-2 and IL-17 (Duarte et al., 2009; Komatsu et al., 2009). $FoxP3^+$ T cells may acquire T_H cell features without losing FoxP3 expression. These hybrid $FoxP3^+ROR\gamma t^+IL\text{-}17^+$ or $FoxP3^+T\text{-}bet^+IFN\text{-}\gamma^+$ cells have been characterized as in a transitional state between Treg and T_H phenotypes (Oldenhove et al., 2009). The issue of Treg plasticity is

controversial and it remains to be established whether it reflects a true lineage reprogramming of committed Tregs or is a dynamic transition of an uncommitted population of FoxP3$^+$ T cells (Hori, 2010). This high degree of plasticity in the iTreg developmental program represents an additional challenge to the inherent difficulties associated with the study of human Tregs, such as insufficient number of cells, heterogeneous cell population and staggering differences between human and mouse models, which is particularly important when thinking about using Tregs as a therapeutic treatment for human disease. These intrinsic limitations have prevented a comprehensive understanding of the differential signaling events that govern the development and function of Tregs.

Inhibitory effect of AKT on Tregs

In 2008 Sauer et al. (Sauer et al., 2008) reported that the PI3K/AKT activity antagonized FoxP3 expression in murine T cells. The involvement of the AKT pathway was further suggested when a constitutively active AKT repressed the TGF-β induction of FoxP3 in a rapamycin-sensitive manner. In addition, the combined inhibition of both, PI3K and mTOR, greatly augmented FoxP3 induction in CD4$^+$ T cells (Sauer et al., 2008). Additionally, only a moderate direct inhibition of AKT sufficed to increase FoxP3 expression in T cells, conveying that the blockage of AKT itself promoted FoxP3 expression rather than an upstream regulator. In a comparison of PI3K/mTOR inhibitor-induced cells to freshly isolated Treg cells, more than half of the transcripts specifically regulated in Treg cells were similarly regulated in FoxP3-induced cells via PI3K/mTOR inhibition, suggesting a substantial association between suppression of the PI3K/AKT pathway, *de novo* expression of FoxP3 and Treg signature in mouse CD4$^+$ T cells (Sauer et al., 2008). The involvement of AKT in Treg development was further investigated by Haxhinasto et al. (2008), who reported that if FoxP3 expression was already established in T cells, the constitutive activation of AKT did not significantly decrease its expression. The authors also investigated the role of AKT in the development of nTregs and concluded that, like iTregs, activation of AKT significantly impaired thymic CD4$^+$FoxP3$^+$ nTreg development without altering positive selection of conventional CD4$^+$ and CD8$^+$ T cells. These results placed AKT at a nexus of signaling pathways with broad impact on the onset of Treg specification in thymus as well as on the differentiation of peripheral iTregs.

In addition to the critical role of AKT on Treg development, recent reports established the negative impact of AKT on the suppressive function of human nTregs. Crellin et al. (Crellin et al., 2007) investigated whether hyporesponsiveness and lack of cytokine production in Tregs compared to conventional T cells was the result of altered TCR signaling. The authors revealed that CD4$^+$CD25$^-$ T cells and CD4$^+$CD25$^+$ Tregs had equivalent levels of ERK1/2 and p38 phosphorylation upon TCR stimulation. However, CD4$^+$CD25$^+$ Treg cells displayed a consistent defect in phosphorylation of AKT at S473 and T308 as well as reduced phosphorylation of the AKT substrates FoxO and S6, suggesting a specific blockade of the TCR-induced AKT pathway. Moreover, constitutive activation of AKT in nTregs led to increased proliferation and concomitant abrogated suppressive capacity, further supporting the requirement of a "silent" AKT to sustain Treg function. In addition, enhanced AKT activity in CD4$^+$CD25$^+$ nTreg cells did not suppress expression of FoxP3, CTLA-4 or CD25 which indicated that the loss of function was not related to a phenotypic change (Crellin et al., 2007). In conclusion, these seminal studies demonstrated the connection between biologic function and the altered AKT signaling pathway in CD4$^+$CD25$^+$ nTreg cells. Unlike the AKT requirement in the conventional T cell effector response, AKT activation in Tregs significantly impairs their development and function. In an effort to understand the mechanisms that regulate the transition from conventional T cells to iTregs, our group has recently reported that the rewiring of the TCR pathway occurring during the iTreg differentiation also results in the specific blockade of the TCR-dependent AKT activation and has elucidated some of the signaling events involved in this altered TCR signaling (Reneer et al., 2011).

The opposing role of AKT on human iTregs

We have developed an *ex vivo* cell culture system that mimics *in vivo* human iTreg development and allows for the discrimination and comparison of naïve, memory and iTreg T cell populations simultaneously isolated from the same original pool of primary T cells [Ellis G I, Reneer M C, et al., *Generation of induced Regulatory T Cells from primary human naïve and memory T cells*. JoVE (in press)]. These iTregs exhibit high levels of CD25, FoxP3, CTLA4, GITR, low levels of CD127, and display strong suppressor activity. Using this novel experimental platform, our laboratory has identified that the differentiation of conventional T cells into iTregs involves the reorganization of TCR-dependent AKT activation. We have confirmed the TCR inability to activate AKT in primary human iTregs like that of mouse Tregs, and have further determined some of the signaling mechanisms involved in this specific blockage (Reneer et al., 2011). In contrast, ERK activation was remarkably effective in response to TCR engagement, indicating that the blockade of the AKT pathway in iTregs did not occur as a result of a general failure of the TCR machinery. When we investigated what was causing this defective TCR response in Tregs, we identified the functional dissociation of the K$^+$-channel Kv1.3 and the kinase Lck from TCR engagement. Interestingly, Kv1.3 and Lck relocate to and participate in the signaling pathway triggered by the highly active IL-2-Receptor (IL-2R) complex. From these results, we propose that the competitive crosstalk between TCR and IL2-R pathway is instrumental in

the signaling rewiring responsible for the blockage of the TCR-dependent AKT activation in iTregs. In addition, the physical and functional redistribution of protein clusters may represent a rapid and efficient mechanism of adapting the cell signaling machinery to a new environmental context. Indeed, the functional switch from a prevalent antigen-dependent signaling in effector cells to cytokine-dependent responsiveness in iTregs is consistent with the physiologic relevance of the suppressor activity of iTregs upon antigen clearance (Reneer et al., 2011).

The AKT network determines effector T cell sensitivity to Treg suppression

The AKT pathway is not only critical for Treg development and function, but is also an essential determinant of effector T cell sensitivity to Treg cell-mediated suppression. In several murine models of spontaneous autoimmunity, such as the non-obese diabetic (NOD) model of diabetes and the MRL/Mp model of systemic lupus erythematosus, Tregs function normally but effector T cells are resistant to Treg-mediated suppression (Gregori et al., 2003; Monk et al., 2005; You et al., 2005). In addition, several strains of mice with altered T cell intracellular signaling were also refractory to iTreg activity (Rengarajan et al., 2002; Bopp et al., 2005). But it was not until King et al. (King et al., 2006) that a potential mechanistic explanation for the effector T cell insensitivity to Tregs was found in the hyperactivation of the PI3K-AKT pathway. Concrete evidence for a causal relationship between AKT and effector T cell resistance was confirmed in a recent study by Wehrens et al. (Wehrens et al., 2011) in human patients with juvenile idiopathic arthritis (JIA). Tregs from synovial fluid and peripheral blood of patients with JIA were fully functional when compared to healthy controls; however, at the local site of autoimmune inflammation, proliferation and cytokine production of effector T cells was poorly controlled by Tregs. These T cells isolated from the site of inflammation had increased levels of active AKT, and the pharmacological inhibition of AKT kinase renders them susceptible to Treg-mediated suppression. These findings demonstrate that not only a weak Treg niche, but also a dysfunctional effector population may account for the attenuated suppressor activity and loss of immune homeostasis (Wehrens et al., 2011). Further evidence for the importance of AKT in the effector T cell interplay with Tregs is the homeostatic control of the maturation of Ag-primed cytotoxic T cell (CTL) precursors to become CTL effector cells by a mechanism that involves the inhibition of AKT, but not of STAT5 or ZAP70 (Kojima et al., 2005).

In conclusion, the role of the AKT pathway has been known for many years to be critical in the development and function of conventional T cells. However, the inhibitory effect of AKT on Treg cell generation and suppressor activity in both mice and humans has just recently been uncovered. Additionally, hyperactivation of AKT in effector T cells leads to the loss of suppressive capacity of Tregs, suggesting that AKT is also crucial for effector cells to tune up their Treg sensitivity. Depicted in Fig. 4 is a summary of the balancing roles that AKT plays in conventional T cells and Tregs.

Perspectives

As the central role of AKT is being characterized in different T cell subpopulations and activation stages, the particular complexity of its regulatory signaling pathway in Tregs is mostly unknown. What are the mechanisms underlying the paucity of AKT activity in Tregs? What is causing the low TCR-dependent AKT inactivation in Tregs? Our laboratory has reported basic differences in the early TCR signaling machinery in iTregs compared to conventional T cells such as the dissociation of Lck and Kv1.3 from the TCR and their relocation to the IL-2R pathway (Reneer et al., 2011). However, more investigation into early TCR signaling events will be needed to fully understand the rewiring of TCR signaling upstream of AKT activation that occurs during iTreg differentiation. What is the contribution of phosphatases such as PTEN, PP2A and PHLPP? This question has been answered partially by the finding that PHLPP is overexpressed in murine Tregs (Patterson et al., 2011), although the confirmation in human models remains elusive as well as the involvement of other phosphatases. What is the specific role of kinases such PDK1 and mTOR in Tregs? What are the mechanisms that control their function? The addition of rapamycin in cell culture favors the generation of these cells. However, rapamycin preferentially inhibits mTORC1 which is not involved in AKT activation (Sauer et al., 2008). A deeper understanding into the activity of kinases in Tregs will help to elucidate the singularity of the AKT signaling in Tregs and will open novel avenues toward therapeutic manipulation of Tregs. Interestingly, our laboratory has shown that the expression of AKT protein was substantially higher in Tregs when compared with conventional T cells (Reneer et al., 2011), probably because of the increased stability of non-active AKT (Suizu et al., 2009). Our results showed that this low-active AKT in iTregs co-immunoprecipitated with SMAD3, as it has been previously reported in other cell types (Conery et al., 2004; Remy et al., 2004; Song et al., 2006), suggesting a new level of cross-talk between the TGF-β and the TCR pathways in iTregs. We propose a model by which the excess of AKT in Tregs may result in the cytoplasmic sequestration of SMAD3 and the prevention of the SMAD3-dependent upregulation of pro-apoptotic factors (manuscript in preparation). We are currently determining how the kinetics and balance of SMAD3-dependent FoxP3 expression fit with AKT's control of SMAD3 activity in iTregs.

Likewise, the investigation into the mechanisms by which overactivation of AKT activity in effector T cells located in inflammatory environments precludes Treg suppression will

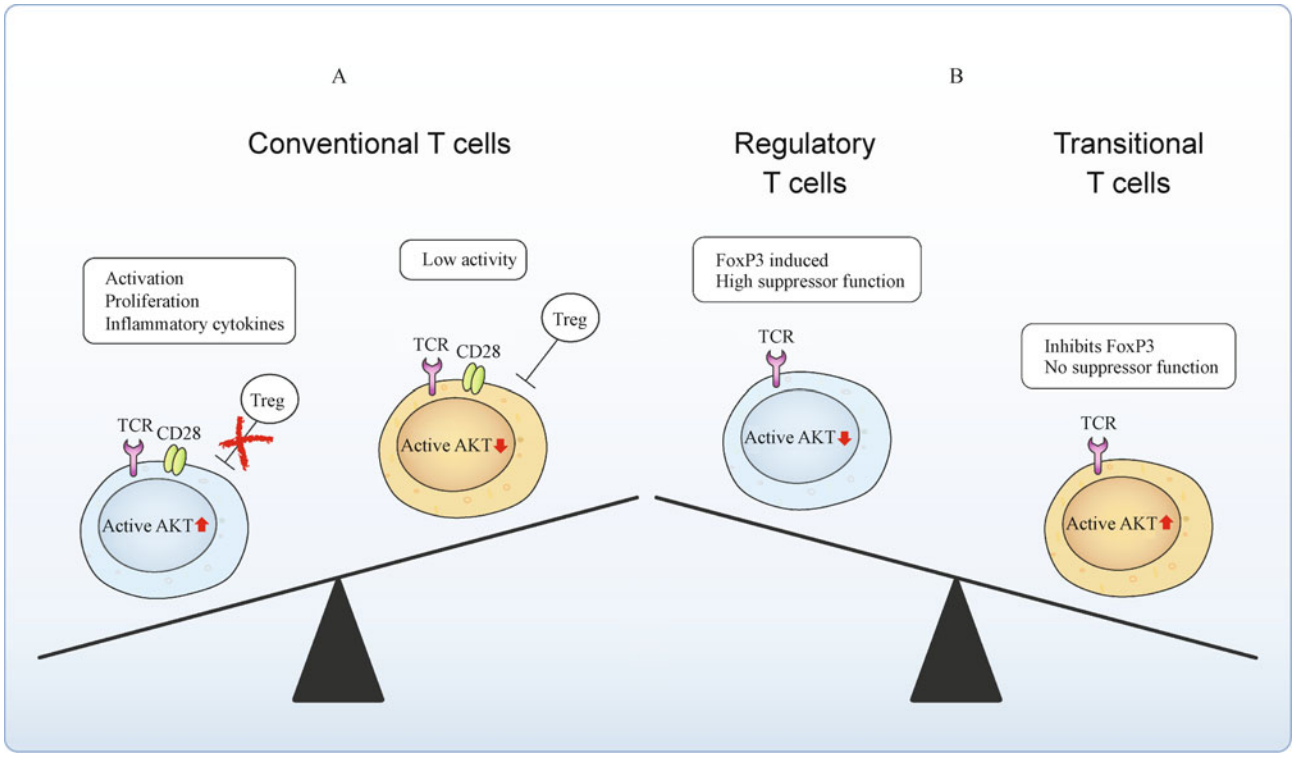

Figure 4 Balancing act of AKT in T cell differentiation and activation. (A): *AKT in conventional T cells*. Ligation of Ag-MHC to the TCR will result in activation of AKT in conventional T cells. AKT activation will subsequently induce proliferation and production of inflammatory cytokines. Additionally, under certain pathological conditions, effector T cells exhibit hyperactivation of the AKT pathway, which will prevent the suppressive action of Tregs. Partial reduction of AKT activity in conventional T cells allows Treg-mediated suppression, which further inactivates the AKT pathway in the targeted cell. (B): *AKT in regulatory T cells*. Treg development and function require reduced AKT activity. In the periphery, the rewiring of the proximal TCR signaling network with the specific blockage of the TCR-induced AKT pathway is a necessary event for iTreg differentiation and acquisition of suppressor capacity. These iTregs inhibit conventional T cells through the downregulation of the AKT pathway. Concurrently, AKT activation prevents FoxP3 expression and inhibits Treg suppressor function. Therefore, high AKT activation tilts the balance of the immune response toward optimal effector function, while lesser AKT signaling in T cells will occur in an anti-inflammatory environment, conductive to preferential Treg differentiation and suppressive function.

shed light on potential new therapeutic targets for the treatment of autoimmune diseases in which effector T cells are unresponsive to Treg control. In the same context, a latent question is how do Tregs subvert the AKT pathway in their target cells? Several mechanisms for Treg suppression have been described and partially characterized, but the signaling events elicited by Treg-dependent activity in the effector cells have not been studied, with the exception of the recognized involvement of AKT. A better understanding of the complexities of the AKT pathway regulation in T cells might help to characterize the seemingly opposite functions it exerts in different T cell subsets, differentiation stages or specific patterns of activation. Answers to these questions are necessary to design novel therapeutic strategies either targeting AKT and/or exploiting the immense potential of Treg manipulation.

Abbreviations

Protein Kinase B (AKT); protein kinase C (PKC); protein kinase A (PKA); phosphatidylinositol 3-kinase (PI3K); cAMP-dependent protein kinase A/protein kinase G/protein kinase C (AGC); pleckstrin homology (PH); hydrophobic motif (HM); phosphatidylinositol (PtdIns); Phosphoinositide-Dependent Kinase-1 (PDK1); mammalian target of rapamycin (mTOR); mTOR complex-1/2 (mTORC1/2); phosphatase and tensin homolog deleted on chromosome 10 (PTEN); SH2-containing inositol polyphosphate 5-phosphatase (SHIP1); PH-like domain, family A, member 1 (PHDLA); PH-like domain family A member 3 (PHLDA3); protein phosphatase 2 (PP2A); PH domain leucine-rich repeat protein phosphatase (PHLPP); Interleukin (IL); IL2-Receptor (IL2-R); Lymphocyte-specific protein tyrosine kinase (Lck); Glucocorticoid-Induced Tumor Necrosis Factor Receptor Related protein (GITR); regulatory T cells (Tregs); natural Tregs (nTregs); induced Tregs (iTregs); helper T cells (T_H cells); early thymic progenitors (ETPs); Double Negative (DN); Double Positive (DP); T Cell Receptor (TCR); C-C chemokine receptor 6 (CCR6); glycogen synthase kinase 3 (GSK-3); nuclear factor of activated T cells (NFAT); eukaryotic initiation factor 2 (eIF-2B); nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB); B cell

lymphoma 2 (Bcl-2); Cyclin-dependent kinase inhibitor 1B ($p27^{kip1}$); Bcl-2-like protein 11 (Bim); Bcl-2-associated death promoter (BAD); cyclic adenosine 3′,5′-monophosphate (cAMP)-responsive element binding protein (CREB); Dendritic Cell (DC); Immunodysregulation Polyendocrinopathy and Enteropathy, X-linked syndrome (IPEX); cytotoxic T lymphocyte antigen 4 (CTLA4); transforming growth factor-β (TGF-β); retinoic acid-related orphan receptor γt (RORγt); non-obese diabetic (NOD); juvenile idiopathic arthritis (JIA); cytotoxic T cell (CTL); mothers against decapentaplegic homolog (SMAD).

Acknowledgements

We thank M. Moliner for assistance with graphics. This work was partially supported by NIH Grant Numbers R03AR052904-02 from the NIAMS and 2P20 RR020171 from the NCRR to F.M. The authors declare no financial or commercial conflict of interest.

Compliance with ethics guidelines

The authors Mary Catherine Reneer and Francesc Marti declare that they have no conflict of interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Akbar A N, Vukmanovic-Stejic M, Taams L S, Macallan D C (2007). The dynamic co-evolution of memory and regulatory CD4+ T cells in the periphery. Nat Rev Immunol, 7(3): 231–237

Alessi D R, Cohen P (1998). Mechanism of activation and function of protein kinase B. Curr Opin Genet Dev, 8(1): 55–62

Alessi D R, Pearce L R, García-Martínez J M (2009). New insights into mTOR signaling: mTORC2 and beyond. Sci Signal, 2(67): pe27

Altomare D A, Guo K, Cheng J Q, Sonoda G, Walsh K, Testa J R (1995). Cloning, chromosomal localization and expression analysis of the mouse Akt2 oncogene. Oncogene, 11(6): 1055–1060

Andjelković M, Jakubowicz T, Cron P, Ming X F, Han J W, Hemmings B A (1996). Activation and phosphorylation of a pleckstrin homology domain containing protein kinase (RAC-PK/PKB) promoted by serum and protein phosphatase inhibitors. Proc Natl Acad Sci USA, 93(12): 5699–5704

Annacker O, Pimenta-Araujo R, Burlen-Defranoux O, Barbosa T C, Cumano A, Bandeira A (2001). CD25+ CD4+ T cells regulate the expansion of peripheral CD4 T cells through the production of IL-10. J Immunol, 166(5): 3008–3018

Apostolou I, Sarukhan A, Klein L, von Boehmer H (2002). Origin of regulcdory T cells with known specificity for antigen. Nat Immunol, 3(8): 756–763

Arimura Y, Shiroki F, Kuwahara S, Kato H, Dianzani U, Uchiyama T, Yagi J (2004). Akt is a neutral amplifier for Th cell differentiation. J Biol Chem, 279(12): 11408–11416

Balendran A, Casamayor A, Deak M, Paterson A, Gaffney P, Currie R, Downes C P, Alessi D R (1999). PDK1 acquires PDK2 activity in the presence of a synthetic peptide derived from the carboxyl terminus of PRK2. Curr Biol, 9(8): 393–404

Beals C R, Sheridan C M, Turck C W, Gardner P, Crabtree G R (1997). Nuclear export of NF-ATc enhanced by glycogen synthase kinase-3. Science, 275(5308): 1930–1933

Bellacosa A, Testa J R, Staal S P, Tsichlis P N (1991). A retroviral oncogene, akt, encoding a serine-threonine kinase containing an SH2-like region. Science, 254(5029): 274–277

Bluestone J A, Abbas A K (2003). Natural versus adaptive regulatory T cells. Nat Rev Immunol, 3(3): 253–257

Bopp T, Palmetshofer A, Serfling E, Heib V, Schmitt S, Richter C, Klein M, Schild H, Schmitt E, Stassen M (2005). NFATc2 and NFATc3 transcription factors play a crucial role in suppression of $CD4^+$ T lymphocytes by $CD4^+$ $CD25^+$ regulatory T cells. J Exp Med, 201(2): 181–187

Brodbeck D, Cron P, Hemmings B A (1999). A human protein kinase Bgamma with regulatory phosphorylation sites in the activation loop and in the C-terminal hydrophobic domain. J Biol Chem, 274(14): 9133–9136

Brognard J, Sierecki E, Gao T, Newton A C (2007). PHLPP and a second isoform, PHLPP2, differentially attenuate the amplitude of Akt signaling by regulating distinct Akt isoforms. Mol Cell, 25(6): 917–931

Brunet A, Bonni A, Zigmond M J, Lin M Z, Juo P, Hu L S, Anderson M J, Arden K C, Blenis J, Greenberg M E (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell, 96(6): 857–868

Brunkow M E, Jeffery E W, Hjerrild K A, Paeper B, Clark L B, Yasayko S A, Wilkinson J E, Galas D, Ziegler S F, Ramsdell F (2001). Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet, 27(1): 68–73

Burgering B M, Coffer P J (1995). Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. Nature, 376 (6541): 599–602

Burgering B M, Kops G J (2002). Cell cycle and death control: long live Forkheads. Trends Biochem Sci, 27(7): 352–360

Cantley L C, Neel B G (1999). New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. Proc Natl Acad Sci USA, 96(8): 4240–4245

Cardone M H, Roy N, Stennicke H R, Salvesen G S, Franke T F, Stanbridge E, Frisch S, Reed J C (1998). Regulation of cell death protease caspase-9 by phosphorylation. Science, 282(5392): 1318–1321

Carpenter C L, Cantley L C (1996). Phosphoinositide kinases. Curr Opin Cell Biol, 8(2): 153–158

Chen C, Edelstein L C, Gélinas C (2000). The Rel/NF-kappaB family directly activates expression of the apoptosis inhibitor Bcl-x(L). Mol Cell Biol, 20(8): 2687–2695

Cheng J Q, Godwin A K, Bellacosa A, Taguchi T, Franke T F, Hamilton T C, Tsichlis P N, Testa J R (1992). AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. Proc Natl Acad Sci USA, 89(19): 9267–9271

Coffer P J, Woodgett J R (1991). Molecular cloning and characterisation of a novel putative protein-serine kinase related to the cAMP-

dependent and protein kinase C families. Eur J Biochem, 201(2): 475–481

Conery A R, Cao Y, Thompson E A, Townsend C M Jr, Ko T C, Luo K (2004). Akt interacts directly with Smad3 to regulate the sensitivity to TGF-beta induced apoptosis. Nat Cell Biol, 6(4): 366–372

Coombes J L, Siddiqui K R, Arancibia-Cárcamo C V, Hall J, Sun C M, Belkaid Y, Powrie F (2007). A functionally specialized population of mucosal $CD103^+$ DCs induces $Foxp3^+$ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med, 204(8): 1757–1764

Crellin N K, Garcia R V, Levings M K (2007). Altered activation of AKT is required for the suppressive function of human $CD4^+CD25^+$ T regulatory cells. Blood, 109(5): 2014–2022

Datta S R, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, Greenberg M E (1997). Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell, 91(2): 231–241

del Peso L, González-García M, Page C, Herrera R, Nuñez G (1997). Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science, 278(5338): 687–689

Du K, Montminy M (1998). CREB is a regulatory target for the protein kinase Akt/PKB. J Biol Chem, 273(49): 32377–32379

Duarte J H, Zelenay S, Bergman M L, Martins A C, Demengeot J (2009). Natural Treg cells spontaneously differentiate into pathogenic helper cells in lymphopenic conditions. Eur J Immunol, 39(4): 948–955

Dummler B, Hemmings B A (2007). Physiological roles of PKB/Akt isoforms in development and disease. Biochem Soc Trans, 35(2): 231–235

Feuerer M, Hill J A, Mathis D, Benoist C (2009). $Foxp3^+$ regulatory T cells: differentiation, specification, subphenotypes. Nat Immunol, 10 (7): 689–695

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

Franke T F, Yang S I, Chan T O, Datta K, Kazlauskas A, Morrison D K, Kaplan D R, Tsichlis P N (1995). The protein kinase encoded by the Akt proto-oncogene is a target of the PDGF-activated phosphatidylinositol 3-kinase. Cell, 81(5): 727–736

Gao T, Furnari F, Newton A C (2005). PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. Mol Cell, 18(1): 13–24

Godfrey D I, Kennedy J, Suda T, Zlotnik A (1993). A developmental pathway involving four phenotypically and functionally distinct subsets of CD3-CD4-CD8- triple-negative adult mouse thymocytes defined by CD44 and CD25 expression. J Immunol, 150(10): 4244–4252

Gregori S, Giarratana N, Smiroldo S, Adorini L (2003). Dynamics of pathogenic and suppressor T cells in autoimmune diabetes development. J Immunol, 171(8): 4040–4047

Hagenbeek T J, Naspetti M, Malergue F, Garçon F, Nunès J A, Cleutjens K B, Trapman J, Krimpenfort P, Spits H (2004). The loss of PTEN allows TCR alphabeta lineage thymocytes to bypass IL-7 and Pre-TCR-mediated signaling. J Exp Med, 200(7): 883–894

Hanada M, Feng J, Hemmings B A (2004). Structure, regulation and function of PKB/AKT—a major therapeutic target. Biochim Biophys Acta, 1697(1-2): 3–16

Harrington L S, Findlay G M, Lamb R F (2005). Restraining PI3K: mTOR signalling goes back to the membrane. Trends Biochem Sci, 30(1): 35–42

Haxhinasto S, Mathis D, Benoist C (2008). The AKT-mTOR axis regulates de novo differentiation of CD4 + Foxp3 + cells. J Exp Med, 205(3): 565–574

Hill M M, Andjelkovic M, Brazil D P, Ferrari S, Fabbro D, Hemmings B A (2001). Insulin-stimulated protein kinase B phosphorylation on Ser-473 is independent of its activity and occurs through a staurosporine-insensitive kinase. J Biol Chem, 276(28): 25643–25646

Hinton H J, Alessi D R, Cantrell D A (2004). The serine kinase phosphoinositide-dependent kinase 1 (PDK1) regulates T cell development. Nat Immunol, 5(5): 539–545

Hirai H, Sootome H, Nakatsuru Y, Miyama K, Taguchi S, Tsujioka K, Ueno Y, Hatch H, Majumder P K, Pan B S, Kotani H (2010). MK-2206, an allosteric Akt inhibitor, enhances antitumor efficacy by standard chemotherapeutic agents or molecular targeted drugs *in vitro* and *in vivo*. Mol Cancer Ther, 9(7): 1956–1967

Hoffman K, Holmes F A, Fraschini G, Esparza L, Frye D, Raber M N, Newman R A, Hortobagyi G N (1996). Phase I-II study: triciribine (tricyclic nucleoside phosphate) for metastatic breast cancer. Cancer Chemother Pharmacol, 37(3): 254–258

Hori S (2010). Developmental plasticity of $Foxp3^+$ regulatory T cells. Curr Opin Immunol, 22(5): 575–582

Hori S, Nomura T, Sakaguchi S (2003). Control of regulatory T cell development by the transcription factor Foxp3. Science, 299(5609): 1057–1061

Horwitz D A, Zheng S G, Gray J D (2008). Natural and TGF-beta-induced Foxp3(+)CD4(+) CD25(+) regulatory T cells are not mirror images of each other. Trends Immunol, 29(9): 429–435

Huang J, Manning B D (2009). A complex interplay between Akt, TSC2 and the two mTOR complexes. Biochem Soc Trans, 37(1): 217–222

Jefferies H B, Fumagalli S, Dennis P B, Reinhard C, Pearson R B, Thomas G (1997). Rapamycin suppresses 5'TOP mRNA translation through inhibition of p70s6k. EMBO J, 16(12): 3693–3704

Jones P F, Jakubowicz T, Pitossi F J, Maurer F, Hemmings B A (1991). Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. Proc Natl Acad Sci USA, 88(10): 4171–4175

Josefowicz S Z, Rudensky A (2009). Control of regulatory T cell lineage commitment and maintenance. Immunity, 30(5): 616–625

Juntilla M M, Koretzky G A (2008). Critical roles of the PI3K/Akt signaling pathway in T cell development. Immunol Lett, 116(2): 104–110

Juntilla M M, Wofford J A, Birnbaum M J, Rathmell J C, Koretzky G A (2007). Akt1 and Akt2 are required for alphabeta thymocyte survival and differentiation. Proc Natl Acad Sci USA, 104(29): 12105–12110

Kandel E S, Hay N (1999). The regulation and activities of the multifunctional serine/threonine kinase Akt/PKB. Exp Cell Res, 253 (1): 210–229

Kane L P, Andres P G, Howland K C, Abbas A K, Weiss A (2001). Akt provides the CD28 costimulatory signal for up-regulation of IL-2 and IFN-gamma but not TH2 cytokines. Nat Immunol, 2(1): 37–44

Kane L P, Shapiro V S, Stokoe D, Weiss A (1999). Induction of NF-kappaB by the Akt/PKB kinase. Curr Biol, 9(11): 601–604

Kawase T, Ohki R, Shibata T, Tsutsumi S, Kamimura N, Inazawa J, Ohta T, Ichikawa H, Aburatani H, Tashiro F, Taya Y (2009). PH domain-only protein PHLDA3 is a p53-regulated repressor of Akt. Cell, 136

(3): 535–550

Khattri R, Cox T, Yasayko S A, Ramsdell F (2003). An essential role for Scurfin in $CD4^+CD25^+$ T regulatory cells. Nat Immunol, 4(4): 337–342

Khoshnan A, Tindell C, Laux I, Bae D, Bennett B, Nel A E (2000). The NF-kappa B cascade is important in Bcl-xL expression and for the anti-apoptotic effects of the CD28 receptor in primary human $CD4^+$ lymphocytes. J Immunol, 165(4): 1743–1754

King C G, Kobayashi T, Cejas P J, Kim T, Yoon K, Kim G K, Chiffoleau E, Hickman S P, Walsh P T, Turka L A, Choi Y (2006). TRAF6 is a T cell-intrinsic negative regulator required for the maintenance of immune homeostasis. Nat Med, 12(9): 1088–1092

Kleijn M, Scheper G C, Voorma H O, Thomas A A (1998). Regulation of translation initiation factors by signal transduction. Eur J Biochem, 253(3): 531–544

Kojima H, Kanno Y, Hase H, Kobata T (2005). $CD4^+CD25^+$ regulatory T cells attenuate the phosphatidylinositol 3-kinase/Akt pathway in antigen-primed immature CD8+ CTLs during functional maturation. J Immunol, 174(10): 5959–5967

Komatsu N, Mariotti-Ferrandiz M E, Wang Y, Malissen B, Waldmann H, Hori S (2009). Heterogeneity of natural Foxp3+ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity. Proc Natl Acad Sci USA, 106(6): 1903–1908

Konishi H, Kuroda S, Tanaka M, Matsuzaki H, Ono Y, Kameyama K, Haga T, Kikkawa U (1995). Molecular cloning and characterization of a new member of the RAC protein kinase family: association of the pleckstrin homology domain of three types of RAC protein kinase with protein kinase C subspecies and beta gamma subunits of G proteins. Biochem Biophys Res Commun, 216(2): 526–534

Lafont V, Astoul E, Laurence A, Liautard J, Cantrell D (2000). The T cell antigen receptor activates phosphatidylinositol 3-kinase-regulated serine kinases protein kinase B and ribosomal S6 kinase 1. FEBS Lett, 486(1): 38–42

Lee K, Gudapati P, Dragovic S, Spencer C, Joyce S, Killeen N, Magnuson M A, Boothby M (2010). Mammalian target of rapamycin protein complex 2 regulates differentiation of Th1 and Th2 cell subsets via distinct signaling pathways. Immunity, 32(6): 743–753

Levelt C N, Carsetti R, Eichmann K (1993a). Regulation of thymocyte development through CD3. II. Expression of T cell receptor beta CD3 epsilon and maturation to the $CD4^+8^+$ stage are highly correlated in individual thymocytes. J Exp Med, 178(6): 1867–1875

Levelt C N, Ehrfeld A, Eichmann K (1993b). Regulation of thymocyte development through CD3. I. Timepoint of ligation of CD3 epsilon determines clonal deletion or induction of developmental program. J Exp Med, 177(3): 707–716

Liao Y, Hung M C (2004). A new role of protein phosphatase 2a in adenoviral E1A protein-mediated sensitization to anticancer drug-induced apoptosis in human breast cancer cells. Cancer Res, 64(17): 5938–5942

Livolsi A, Busuttil V, Imbert V, Abraham R T, Peyron J F (2001). Tyrosine phosphorylation-dependent activation of NF-kappa B. Requirement for p56 LCK and ZAP-70 protein tyrosine kinases. Eur J Biochem, 268(5): 1508–1515

Madrid L V, Wang C Y, Guttridge D C, Schottelius A J, Baldwin A S Jr, Mayo M W (2000). Akt suppresses apoptosis by stimulating the transactivation potential of the RelA/p65 subunit of NF-kappaB. Mol Cell Biol, 20(5): 1626–1638

Maira S M, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chène P, De Pover A, Schoemaker K, Fabbro D, Gabriel D, Simonen M, Murphy L, Finan P, Sellers W, García-Echeverría C (2008). Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent *in vivo* antitumor activity. Mol Cancer Ther, 7(7): 1851–1863

Manning B D, Cantley L C (2007). AKT/PKB signaling: navigating downstream. Cell, 129(7): 1261–1274

Mao C, Tili E G, Dose M, Haks M C, Bear S E, Maroulakou I, Horie K, Gaitanaris G A, Fidanza V, Ludwig T, Wiest D L, Gounari F, Tsichlis P N (2007). Unequal contribution of Akt isoforms in the double-negative to double-positive thymocyte transition. J Immunol, 178(9): 5443–5453

Markman B, Dienstmann R, Tabernero J (2010). Targeting the PI3K/Akt/mTOR pathway—beyond rapalogs. Oncotarget, 1(7): 530–543

Marone R, Cmiljanovic V, Giese B, Wymann M P (2008). Targeting phosphoinositide 3-kinase: moving towards therapy. Biochim Biophys Acta, 1784(1): 159–185

Mendoza M C, Blenis J (2007). PHLPPing it off: phosphatases get in the Akt. Mol Cell, 25(6): 798–800

Monk C R, Spachidou M, Rovis F, Leung E, Botto M, Lechler R I, Garden O A (2005). MRL/Mp $CD4^+$,$CD25^-$ T cells show reduced sensitivity to suppression by $CD4^+$,$CD25^+$ regulatory T cells *in vitro*: a novel defect of T cell regulation in systemic lupus erythematosus. Arthritis Rheum, 52(4): 1180–1184

Nakatani K, Sakaue H, Thompson D A, Weigel R J, Roth R A (1999). Identification of a human Akt3 (protein kinase B gamma) which contains the regulatory serine phosphorylation site. Biochem Biophys Res Commun, 257(3): 906–910

O'Reilly K E, Rojo F, She Q B, Solit D, Mills G B, Smith D, Lane H, Hofmann F, Hicklin D J, Ludwig D L, Baselga J, Rosen N (2006). mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt. Cancer Res, 66(3): 1500–1508

Obata T, Yaffe M B, Leparc G G, Piro E T, Maegawa H, Kashiwagi A, Kikkawa R, Cantley L C (2000). Peptide and protein library screening defines optimal substrate motifs for AKT/PKB. J Biol Chem, 275(46): 36108–36115

Oldenhove G, Bouladoux N, Wohlfert E A, Hall J A, Chou D, Dos Santos L, O'Brien S, Blank R, Lamb E, Natarajan S, Kastenmayer R, Hunter C, Grigg M E, Belkaid Y (2009). Decrease of Foxp3 + Treg cell number and acquisition of effector cell phenotype during lethal infection. Immunity, 31(5): 772–786

Ozes O N, Mayo L D, Gustin J A, Pfeffer S R, Pfeffer L M, Donner D B (1999). NF-kappaB activation by tumour necrosis factor requires the Akt serine-threonine kinase. Nature, 401(6748): 82–85

Padmanabhan S, Mukhopadhyay A, Narasimhan S D, Tesz G, Czech M P, Tissenbaum H A (2009). A PP2A regulatory subunit regulates C. elegans insulin/IGF-1 signaling by modulating AKT-1 phosphorylation. Cell, 136(5): 939–951

Pal S K, Reckamp K, Yu H, Figlin R A (2010). Akt inhibitors in clinical development for the treatment of cancer. Expert Opin Investig Drugs, 19(11): 1355–1366

Papiernik M, de Moraes M L, Pontoux C, Vasseur F, Pénit C (1998). Regulatory CD4 T cells: expression of IL-2R alpha chain, resistance to clonal deletion and IL-2 dependency. Int Immunol, 10(4): 371–

378

Parry R V, Reif K, Smith G, Sansom D M, Hemmings B A, Ward S G (1997). Ligation of the T cell co-stimulatory receptor CD28 activates the serine-threonine protein kinase protein kinase B. Eur J Immunol, 27(10): 2495–2501

Patra A K, Drewes T, Engelmann S, Chuvpilo S, Kishi H, Hünig T, Serfling E, Bommhardt U H (2006). PKB rescues calcineurin/NFAT-induced arrest of Rag expression and pre-T cell differentiation. J Immunol, 177(7): 4567–4576

Patterson S J, Han J M, Garcia R, Assi K, Gao T, O'Neill A, Newton A C, Levings M K (2011). Cutting edge: PHLPP regulates the development, function, and molecular signaling pathways of regulatory T cells. J Immunol, 186(10): 5533–5537

Peifer C, Alessi D R (2008). Small-molecule inhibitors of PDK1. ChemMedChem, 3(12): 1810–1838

Penit C, Vasseur F (1989). Cell proliferation and differentiation in the fetal and early postnatal mouse thymus. J Immunol, 142(10): 3369–3377

Pillai V, Karandikar N J (2007). Human regulatory T cells: a unique, stable thymic subset or a reversible peripheral state of differentiation? Immunol Lett, 114(1): 9–15

Pim D, Massimi P, Dilworth S M, Banks L (2005). Activation of the protein kinase B pathway by the HPV-16 E7 oncoprotein occurs through a mechanism involving interaction with PP2A. Oncogene, 24(53): 7830–7838

Rao A, Luo C, Hogan P G (1997). Transcription factors of the NFAT family: regulation and function. Annu Rev Immunol, 15(1): 707–747

Redpath N T, Foulstone E J, Proud C G (1996). Regulation of translation elongation factor-2 by insulin via a rapamycin-sensitive signalling pathway. EMBO J, 15(9): 2291–2297

Reid J M, Walden C A, Qin R, Ziegler K L, Haslam J L, Rajewski R A, Warndahl R, Fitting C L, Boring D, Szabo E, Crowell J, Perloff M, Jong L, Bauer B A, Mandrekar S J, Ames M M, Limburg P J, and the Cancer Prevention Network (2011). Phase 0 clinical chemoprevention trial of the Akt inhibitor SR13668. Cancer Prev Res (Phila), 4(3): 347–353

Reif K, Burgering B M, Cantrell D A (1997). Phosphatidylinositol 3-kinase links the interleukin-2 receptor to protein kinase B and p70 S6 kinase. J Biol Chem, 272(22): 14426–14433

Remy I, Montmarquette A, Michnick S W (2004). PKB/Akt modulates TGF-beta signalling through a direct interaction with Smad3. Nat Cell Biol, 6(4): 358–365

Reneer M C, Estes D J, Velez-Ortega A C, Norris A, Mayer M, Marti F (2011). Peripherally induced human regulatory T cells uncouple Kv1.3 activation from TCR-associated signaling. Eur J Immunol, 41 (11):3170–3175

Rengarajan J, Tang B, Glimcher L H (2002). NFATc2 and NFATc3 regulate T(H)2 differentiation and modulate TCR-responsiveness of naïve T(H)cells. Nat Immunol, 3(1): 48–54

Rocher G, Letourneux C, Lenormand P, Porteu F (2007). Inhibition of B56-containing protein phosphatase 2As by the early response gene IEX-1 leads to control of Akt activity. J Biol Chem, 282(8): 5468–5477

Roget K, Malissen M, Malbec O, Malissen B, Daëron M (2008). Non-T cell activation linker promotes mast cell survival by dampening the recruitment of SHIP1 by linker for activation of T cells. J Immunol, 180(6): 3689–3698

Romashkova J A, Makarov S S (1999). NF-kappaB is a target of AKT in anti-apoptotic PDGF signalling. Nature, 401(6748): 86–90

Rondinone C M, Carvalho E, Wesslau C, Smith U P (1999). Impaired glucose transport and protein kinase B activation by insulin, but not okadaic acid, in adipocytes from subjects with Type II diabetes mellitus. Diabetologia, 42(7): 819–825

Rong S B, Hu Y, Enyedy I, Powis G, Meuillet E J, Wu X, Wang R, Wang S, Kozikowski A P (2001). Molecular modeling studies of the Akt PH domain and its interaction with phosphoinositides. J Med Chem, 44(6): 898–908

Salomon B, Bluestone J A (2001). Complexities of CD28/B7: CTLA-4 costimulatory pathways in autoimmunity and transplantation. Annu Rev Immunol, 19(1): 225–252

Sarbassov D D, Guertin D A, Ali S M, Sabatini D M (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science, 307(5712): 1098–1101

Sasaki T, Irie-Sasaki J, Jones R G, Oliveira-dos-Santos A J, Stanford W L, Bolon B, Wakeham A, Itie A, Bouchard D, Kozieradzki I, Joza N, Mak T W, Ohashi P S, Suzuki A, Penninger J M (2000). Function of PI3Kgamma in thymocyte development, T cell activation, and neutrophil migration. Science, 287(5455): 1040–1046

Sauer S, Bruno L, Hertweck A, Finlay D, Leleu M, Spivakov M, Knight Z A, Cobb B S, Cantrell D, O'Connor E, Shokat K M, Fisher A G, Merkenschlager M (2008). T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. Proc Natl Acad Sci USA, 105 (22): 7797–7802

Shevach E M (2001). Certified professionals: CD4(+)CD25(+) suppressor T cells. J Exp Med, 193(11): F41–F46

Shevach E M (2009). Mechanisms of foxp3+ T regulatory cell-mediated suppression. Immunity, 30(5): 636–645

Shimoke K, Chiba H (2001). Nerve growth factor prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced cell death via the Akt pathway by suppressing caspase-3-like activity using PC12 cells: relevance to therapeutical application for Parkinson's disease. J Neurosci Res, 63(5): 402–409

Sizemore N, Lerner N, Dombrowski N, Sakurai H, Stark G R (2002). Distinct roles of the Ikappa B kinase alpha and beta subunits in liberating nuclear factor kappa B (NF-kappa B) from Ikappa B and in phosphorylating the p65 subunit of NF-kappa B. J Biol Chem, 277 (6): 3863–3869

Song G, Ouyang G, Bao S (2005). The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med, 9(1): 59–71

Song K, Wang H, Krebs T L, Danielpour D (2006). Novel roles of Akt and mTOR in suppressing TGF-beta/ALK5-mediated Smad3 activation. EMBO J, 25(1): 58–69

Staal S P (1987). Molecular cloning of the akt oncogene and its human homologues AKT1 and AKT2: amplification of AKT1 in a primary human gastric adenocarcinoma. Proc Natl Acad Sci USA, 84(14): 5034–5037

Staal S P, Hartley J W, Rowe W P (1977). Isolation of transforming murine leukemia viruses from mice with a high incidence of spontaneous lymphoma. Proc Natl Acad Sci USA, 74(7): 3065–3067

Stahl M, Dijkers P F, Kops G J, Lens S M, Coffer P J, Burgering B M, Medema R H (2002). The forkhead transcription factor FoxO regulates transcription of p27Kip1 and Bim in response to IL-2. J Immunol, 168(10): 5024–5031

Stambolic V, Suzuki A, de la Pompa J L, Brothers G M, Mirtsos C,

Sasaki T, Ruland J, Penninger J M, Siderovski D P, Mak T W (1998). Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell, 95(1): 29–39

Starr T K, Jameson S C, Hogquist K A (2003). Positive and negative selection of T cells. Annu Rev Immunol, 21(1): 139–176

Suizu F, Hiramuki Y, Okumura F, Matsuda M, Okumura A J, Hirata N, Narita M, Kohno T, Yokota J, Bohgaki M, Obuse C, Hatakeyama S, Obata T, Noguchi M (2009). The E3 ligase TTC3 facilitates ubiquitination and degradation of phosphorylated Akt. Dev Cell, 17(6): 800–810

Tang Q, Bluestone J A (2008). The Foxp3^{+} regulatory T cell: a jack of all trades, master of regulation. Nat Immunol, 9(3): 239–244

Thomas C C, Deak M, Alessi D R, van Aalten D M (2002). High-resolution structure of the pleckstrin homology domain of protein kinase b/akt bound to phosphatidylinositol (3,4,5)-trisphosphate. Curr Biol, 12(14): 1256–1262

Turinsky J, Damrau-Abney A (1998). Akt1 kinase and dynamics of insulin resistance in denervated muscles *in vivo*. Am J Physiol, 275(5 Pt 2): R1425–R1430

Vignali D A, Collison L W, Workman C J (2008). How regulatory T cells work. Nat Rev Immunol, 8(7): 523–532

Vlahos C J, Matter W F, Hui K Y, Brown R F (1994). A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). J Biol Chem, 269(7): 5241–5248

Vukmanovic-Stejic M, Zhang Y, Cook J E, Fletcher J M, McQuaid A, Masters J E, Rustin M H, Taams L S, Beverley P C, Macallan D C, Akbar A N (2006). Human CD4^{+} CD25^{hi} Foxp3^{+} regulatory T cells are derived by rapid turnover of memory populations *in vivo*. J Clin Invest, 116(9): 2423–2433

Wan Q, Kozhaya L, ElHed A, Ramesh R, Carlson T J, Djuretic I M, Sundrud M S, Unutmaz D (2011). Cytokine signals through PI-3 kinase pathway modulate Th17 cytokine production by CCR6 + human memory T cells. J Exp Med, 208(9): 1875–1887

Wehrens E J, Mijnheer G, Duurland C L, Klein M, Meerding J, van Loosdregt J, de Jager W, Sawitzki B, Coffer PJ, Vastert B, Prakken B J, van Wijk F (2011). Functional human regulatory T cells fail to control autoimmune inflammation due to PKB/c-akt hyperactivation in effector cells. Blood, 118(13):3538–3548

Werlen G, Hausmann B, Naeher D, Palmer E (2003). Signaling life and death in the thymus: timing is everything. Science, 299(5614): 1859–1863

Wiesinger D, Gubler H U, Haefliger W, Hauser D (1974). Antiinflammatory activity of the new mould metabolite 11-desacetoxywortmannin and of some of its derivatives. Experientia, 30(2): 135–136

Workman C J, Szymczak-Workman A L, Collison L W, Pillai M R, Vignali D A (2009). The development and function of regulatory T cells. Cell Mol Life Sci, 66(16): 2603–2622

Xu L, Kitani A, Fuss I, Strober W (2007). Cutting edge: regulatory T cells induce CD4^{+}CD25^{-}Foxp3^{-} T cells or are self-induced to become Th17 cells in the absence of exogenous TGF-beta. J Immunol, 178(11): 6725–6729

Yang X O, Nurieva R, Martinez G J, Kang H S, Chung Y, Pappu B P, Shah B, Chang S H, Schluns K S, Watowich S S, Feng X H, Jetten A M, Dong C (2008). Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity, 29(1): 44–56

Yang Z Z, Tschopp O, Baudry A, Dümmler B, Hynx D, Hemmings B A (2004). Physiological functions of protein kinase B/Akt. Biochem Soc Trans, 32(2): 350–354

You S, Belghith M, Cobbold S, Alyanakian M A, Gouarin C, Barriot S, Garcia C, Waldmann H, Bach J F, Chatenoud L (2005). Autoimmune diabetes onset results from qualitative rather than quantitative age-dependent changes in pathogenic T-cells. Diabetes, 54(5): 1415–1422

Yung H W, Charnock-Jones D S, Burton G J (2011). Regulation of AKT phosphorylation at Ser473 and Thr308 by endoplasmic reticulum stress modulates substrate specificity in a severity dependent manner. PLoS ONE, 6(3): e17894

Zhou L, Lopes J E, Chong M M, Ivanov I I, Min R, Victora G D, Shen Y, Du J, Rubtsov Y P, Rudensky A Y, Ziegler S F, Littman D R (2008). TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature, 453(7192): 236–240

Ziegler S F (2006). FOXP3: of mice and men. Annu Rev Immunol, 24 (1): 209–226