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Functional role of Akt in macrophage-mediated innate immunity

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1. ABSTRACT

Akt (protein kinase B) is a serine/threonine protein kinase that regulates cell metabolism, survival and proliferation. Recent studies of the role of Akt in phagocytosis, intracellular bacterial infections, LPS tolerance, production of inflammatory cytokines and mediators, and migration during macrophage-mediated innate immunity strongly suggest a pivotal role for this enzyme in the functional activation of macrophages. Considering that a variety of inflammatory diseases, such as rheumatoid arthritis, atherosclerosis, diabetes, obesity, cancer and osteoporosis, are regulated by macrophage-mediated innate immunity, efforts should be more carefully focused on understanding the function of Akt in macrophage-mediated innate immunity. Although few studies have addressed this question, this review discusses recent findings that support an important role for Akt in macrophage-mediated innate immunity and underlines the need for trials to develop pharmaceutically useful drugs that target Akt for treatment of macrophage-mediated inflammatory diseases. The findings we review here suggest that a novel and safe Akt inhibitor with strong immunosuppressive and anti-inflammatory properties will be applied to various chronic inflammatory diseases in the near future.

2. INTRODUCTION

The immune response is a critical defense mechanism that involves neutralizing, removing, and destroying foreign materials (1). The immune system can be divided into two components, the innate immune system and the adaptive immune system. The innate immune system has several layers of defense: 1) anatomic barriers such as the skin and mucosal surfaces; 2) physiologic barriers such as temperature, pH, and chemical mediators (i.e., lysozyme and collectin); 3) phagocytic/endocytic barriers to foreign organisms and materials; and 4) resistance to infection through the inflammatory response (2). Adaptive immunity, also called acquired immunity, can be divided into the humoral immune response, which is characterized by B cell-mediated antibody production, and the cell-mediated immune response, which is characterized by CD4+ and CD8+ T cell-mediated immune responses. The adaptive immune response is defined based on four central characteristics: antigenic specificity, diversity, memory, and self/non-self recognition (3).

Macrophages represent a major component of the innate immune system and play roles in anti-cancer, anti-bacterial, and anti-viral immune responses (4). These cells are able to directly or indirectly attack tumor cells, virus-

Table 1. Action of Akt subtypes in macrophages and monocytes

Subtype	Cells	Action of Akt	Ref
Akt1	Macrophages	Endotoxin tolerance by controlling miRNA expression	(71)
	RAW264.7 cells	The suppressive action of VIP on TLR4 expression	(70)
	Macrophages	Promotion of intracellular survival of *Salmonella typhimurium* and *Mycobacterium tuberculosis* by controlling actin dynamics through PAK4, and phagosome-lysosome fusion through the AS160 (also known as TBC1D4)-RAB14 pathway	(60)
	Macrophages	Key elements in osteoclast differentiation from macrophages	(94, 95)
	Monocytes	A positive regulator of monocyte/macrophage survival.	(96)
Akt2	PMA-differentiated THP-1 macrophages Primary monocytes	Control of pressure-dependent phagocytosis	(45)
	Macrophages	Key elements in osteoclast differentiation from macrophages	(94)
	THP-1 cells and mouse peritoneal macrophages	LIMK/Cofilin phosphorylation, which contributed to defects in actin polymerization and chemotaxis	(64)
Akt3		Not fully identified	

infected cells and invading bacteria by releasing cytotoxic molecules such as nitric oxide (NO) and reactive oxygen species (ROS), by producing various pro-inflammatory cytokines (tumor necrosis factor [TNF]-α, interleukin [IL]-1, IL-6) and chemokines (macrophage inflammatory protein 1α, IL-8, monocyte chemoattractant protein-1), or by engulfing target pathogens or infected cells (5-7). The activation of macrophages is thus regarded as one good strategy to enhance the body's defense mechanisms. Considering that the thymus, the organ where T cell maturation and development occurs, decreases in size with age, maintenance of an immune defense system mediated by cells other than T cells, such as macrophages, is critical for elderly people. Macrophage-activating biomaterials are thus being screened for the development of immunostimulatory foods or supplements (8, 9).

The inflammation that occurs during innate immune responses is largely mediated by macrophages. This inflammation is driven by immunopathological events such as the over-production of various pro-inflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-12, and inflammatory mediators, including nitric oxide (NO) and prostaglandin E_2 (PGE_2), which are generated by activated inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 (10, 11). Production of these pro-inflammatory cytokines and inflammatory mediators is dependent upon the activation of pattern recognition receptors (PRRs), such as Toll-like receptor (TLR)-4 and TLR-2, by microbial ligands including lipopolysaccharide (LPS) (12). During the activation of PRRs, a series of intracellular signaling molecules, including mitogen activated protein kinases (MAPKs), serine/threonine protein kinases, and non-receptor type tyrosine kinases, are activated, resulting in the upregulation of inflammatory gene expression by transcription factors such as nuclear factor (NF)-κB and activator protein (AP)-1 (13, 14).

Of the numerous signaling proteins that contribute to a large number of signals, Akt (protein kinase B) acts as a major player in the regulation of metabolism, cell survival, motility, transcription, and cell cycle progress (15, 16). It is a serine/threonine-specific protein kinase family member (EC 2.7.11.1) encoded by the Akt family genes Akt1, Akt2, and Akt3 (Table 1) that are widely distributed in a variety of tissues and cells (16, 17). The main functions of Akt are carried out by Akt1, which is involved in regulating cell survival and controlling the proliferation of many types of cancer (16, 18). Akt2 has been shown to play an important role in the insulin signaling pathway and is required for glucose transport (19). In contrast, the function of Akt3 is not fully understood, although defects in brain development were observed in Akt3 knockout mice (16, 17). The substrates of Akt (Figure 1) indicate its role in various cellular responses. Immune cells express the Akt substrates IκBα kinase (IKK), p47phox, Raf, and p70S6K (17, 20-23). These substrates play a central role in mediating chemotactic migration, NF-κB-mediated pro-inflammatory gene expression, ROS-mediated respiratory burst, and phagocytic uptake (22-27). Together these findings demonstrate that Akt acts as a central player in numerous immunobiological and immunopathological events, including acute and chronic inflammatory diseases such as septic shock and rheumatoid arthritis.

Indeed, Akt has been shown to be highly expressed in immune cells such as macrophages, neutrophils, dendritic cells, natural killer cells, and T and B lymphocytes (28, 29). In particular, the expression and phosphorylation of Akt in macrophages are remarkably up-regulated in response to bacteria or bacteria-derived products such as LPS and peptidoglycan (PGN) (28) (Table 2). Because Akt has been identified as a strong positive regulator of various types of cancers and inflammatory diseases (30), strong and effective inhibition of Akt is considered a therapeutic objective for curing a variety of acute or chronic inflammatory diseases, such as septic shock, rheumatoid arthritis, and atherosclerosis, and other inflammatory diseases including cardiovascular disease, chronic obstructive pulmonary disease, inflammatory bowel disease, liver disease, and asthma (16). Although the functional importance of this enzyme in immune responses has been thoroughly demonstrated, few papers have reviewed its role in immune cells, and in particular, in macrophages. The purpose of this review is to summarize recent findings and describe the putative mechanistic role of Akt in innate immunity. Because macrophages are the key cells that mediate host inflammatory and innate immune responses, this review will focus on results of recent studies of the function of Akt in macrophages.

Table 2. Pattern recognition receptors linked to Akt activation in macrophages and monocytes

PRR	Ligand	Action of activated Akt	Ref
TLR1/2	Pam3CSK4	PI3K-induced Akt phosphorylation	(97)
TLR2 TLR2/6 TLR2/dectin-1	Pam3CSK4, PGN, macrophage-activatinglipopeptide-2, Diacylated bacterial lipoproteins, β-glucan,the mycobacterial glycolipids, and the OspC lipoprotein	Phagocyte polarization. Inhibition of neutrophil apoptosis. NF-κB activation Inflammatory gene (COX-2) expression	(97-101)
TLR3	Double-stranded RNA (dsRNA), Poly(I:C)	IRF-3-induced induction of IFN-β production	(102)
TLR4	LPS	NF-κB activation Inflammatory gene expression	(97)
TLR5	Flagellin	Control of VPAC(2) mRNA induction	(103)
TLR7/8	R-848, loxoribine	NF-κB activation Inflammatory gene (COX-2) expression	(97)
TLR9	CpG-DNA	NF-κB activation Inflammatory gene (COX-2) expression Survival of murine macrophage	(97, 104)
TLR10		Not reported	
TLR11		Not reported	

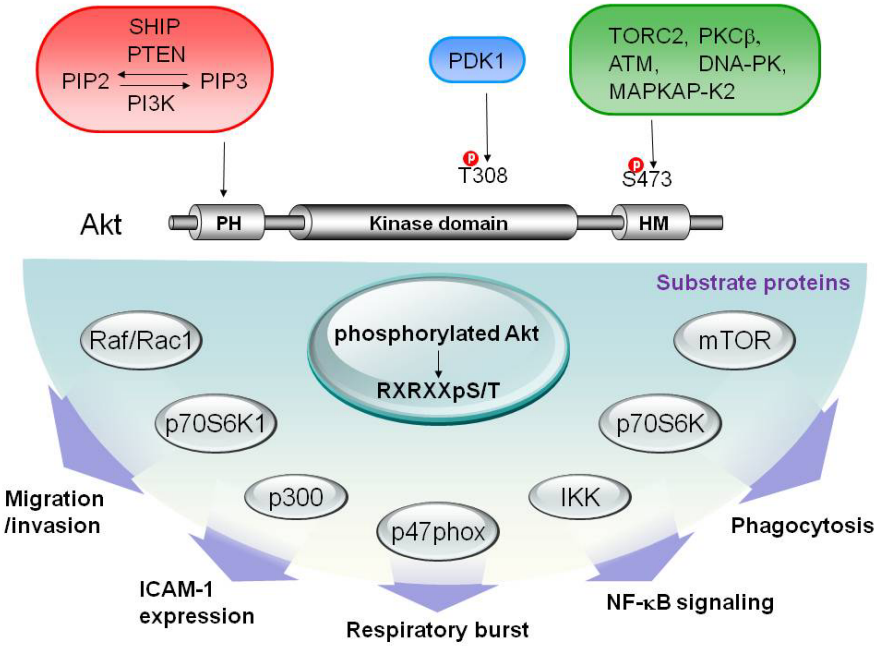

Figure 1. Functional role of Akt managed by various substrate proteins in macrophages and monocytes.

3. STRUCTURE AND FUNCTION OF AKT

3.1. Akt

Akt (Protein kinase B) is expressed as three different isoforms, Akt1, Akt2, and Akt3, which are encoded by distinct genes with similar DNA sequences (17). As shown in Figure 1, members of the Akt family have three functionally distinct domains, the pleckstrin homology (PH) domain, the kinase domain, and the hydrophobic motif (HM) (31). The PH domain plays a critical role in binding phosphatidylinositol-(3,4,5)-triphosphate (PIP3) in the cell membrane, and phosphorylation of the HM is required for Akt activation (18). Although the structural and biochemical features of the three Akt isoforms are very similar, these isoforms perform distinct functions in different organs. Akt1 seems to modulate immune responses, and it has also been reported to be involved in placental development (32). In addition to controlling the growth of adipose tissue, Akt2 is thought to regulate insulin signaling and thus play a role in cellular metabolism (19). Akt3 plays a role in the development of the brain, during which it controls cell number and cell size (16, 17).

3.2. Akt deficiency

Due to the critical functions of each Akt isoform, deficiency in these molecules results in severe growth retardation or lethality (17). Mice deficient in Akt1 indicated a dominant role of Akt1 in the development of placenta and embryo and their maintenance (33, 34). Thus, Akt1 knockout exhibited small placentas and embryos linked to neonatal mortality. The critical causes of this were found as the absence of spongiotrophoblast cells and the reduction of vascularization in mutant placenta (33, 34). Akt2 deficiency developed severe diabetes by suppression of insulin action seen in the case of insulin resistance with hyperglycaemia, hyperinsulinaemia and glucose intolerance (16, 35, 36). In agreement, the mass and number of

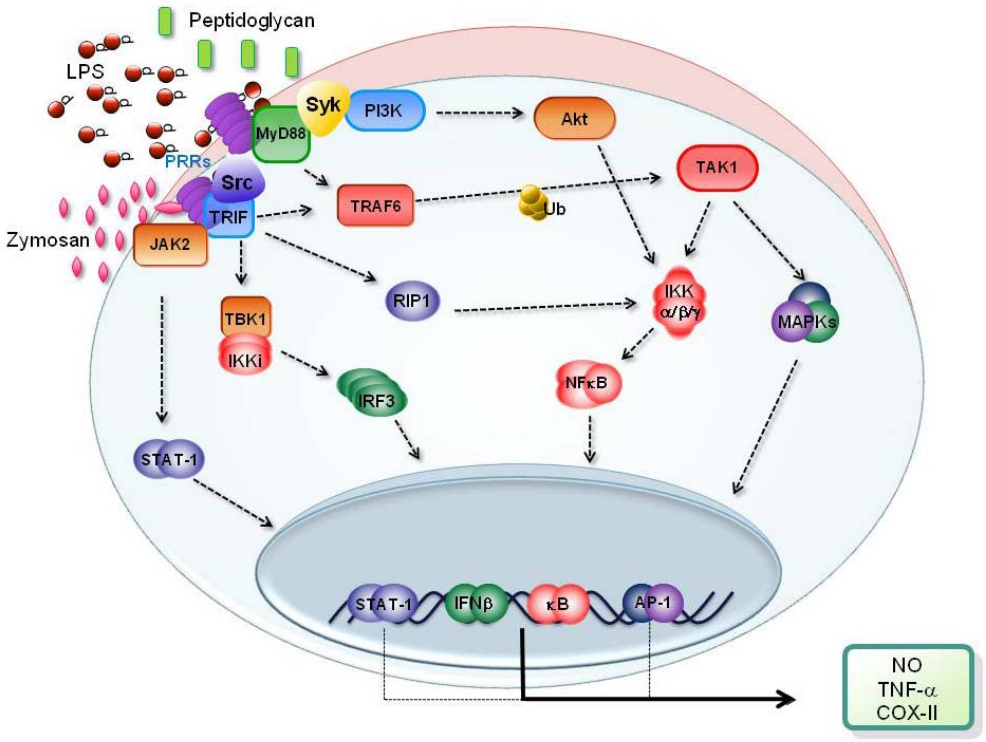

Figure 2. Akt-regulated signaling pathways in inflammatory responses occurred in activated macrophages.

pancreatic islet were greatly increased in Akt knockout mice. Furthermore, the mice exhibited mild growth retardation and an age-dependent loss of adipose tissue (lipoatrophy), suggesting that glucose metabolism, adipogenesis and maintenance, β-cell function and animal growth are critically regulated by Akt2 (15, 37). Defective phenotype of Akt3 knockout condition was seen in brain size and weight without increased perinatal mortality and growth retardation syndrome (15, 34, 38). In adult Akt3 knockout mice, brain size and weight were dramatically reduced with a uniformly reduced brain size, exhibiting proportionally smaller structures (15). These results indicate that the function role of Akt3 could participate in the postnatal development of the brain in mammals.

3.3. Akt substrate proteins

The functions of Akt can be understood by studying its substrate binding capacity. Akt phosphorylates serine or threonine residues within the minimal consensus sequence RxRxx(S/T), in which x is any amino acid, and S/T is the phosphorylation site (17). Many proteins are known to have such sequences. The Akt substrate eNOS is important for angiogenesis (39), WNK1, IRS1, TSC2, GSK3, BRF1, and PLK1 are critical for metabolism (40), IKK, MyK1, Mdm2, p27, p21, Raf, mTOR, and p70S6K are critical for cell growth, protein synthesis, and transcription (41-43), and Bad and Bax are critical for cell survival signaling (44). Some of these Akt substrates are found in macrophage-mediated innate immune responses such as migration, invasion, repiratory burst, inflammatory signaling and phagocytosis as summarized in Figure 1.

3.4. Expression and activity in macrophages

Akt1 and Akt2 are expressed in most cell types and tissues; however, Akt3 expression is largely limited to brain tissue (45). Macrophages and monocytes predominantly express Akt1 and Akt2 but not Akt3 (45). In contrast, most cancer cells have been reported to express all members of the Akt family.

3.5. Akt-mediated activation signaling in macrophages

Two major transcription factors, NF-κB and interferon regulatory factor (IRF)-3, are activated by various TLRs to promote the production of inflammatory mediators such as cytokines and PGE_2 and enhance chemotactic migration and phagocytosis (46). The association of common adaptor proteins such as MyD88, IRAKs (IRAK1 and 4), TRAF6, and TAK1 with the cytoplasmic TIR domain of the Toll like receptors has been shown to be critical for the activation of NF-κB (47). The activation of TAK1 continuously triggers the IKK complex, which consists of IKKα, IKKβ, and NEMO/IKKγ4§. The activated IKK complex in turn phosphorylates IκB, leading to its ubiquitination (49). The resultant degradation of IκB results in the release of bound NF-κB, enabling NF-κB to translocate into the nucleus and induce the expression of inflammatory mediators such as TNF-α, IL-1, iNOS, and COX-2. Akt acts upstream to activate IKK, resulting in activation of the NF-κB pathway. A variety of mitogens and TLR ligands have been reported to activate the Akt/IKK/NF-κB pathway (see Figure 2).

In particular, it is suggested that Akt-mediated NF-κB activation is mainly found to evoke its classical activation pathway, which is managed by MKK3, IKKβ, and NF-κB (p50/p65) for macrophage-mediated innate immunity and inflammatory responses (50).

4. AKT IN MACROPHAGE-MEDIATED INNATE IMMUNITY

4.1. Inflammatory gene expression

Several *in vitro* model systems have been employed to evaluate the mechanisms by which Akt regulates the expression and production of inflammatory genes and mediators. In these models, macrophage-like J774 and RAW264.7 cells or primary peritoneal macrophages were stimulated with bacteria-derived inflammatory stimuli such as LPS and PGN (51-53), and production of various proinflammatory cytokines, including TNF-α, IL-1β, IL-6, and IL-12, and inflammatory mediators, including NO and PGE_2, were measured (54-56).

Most previous reports have focused on the molecular interactions between TLRs and their counter adaptors during inflammatory responses. Some of these studies have reported the cellular location of Akt in TLR-mediated pro-inflammatory signaling cascades. The activation of macrophages by LPS initially requires the molecular association of CD14 and TLR4 to build signaling complexes including adaptor molecules such as MyD88, TRIF, and IRAK and signaling enzymes, such as non-receptor type protein tyrosine kinases [e.g., c-Src and Janus kinase (JAK)-2)], protein kinase C, and protein kinase A (57). Signaling through TLR4-mediated pathways is also associated with the activation of various enzymes, such as nuclear factor (NF)-κB-inducing kinase (NIK), phosphoinositide 3-kinase (PI3K), phosphoinositide-dependent kinase 1 (PDK1), Akt, and MAPKs [such as extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38] (57). These proteins play central roles in controlling several redox-sensitive transcription factors such as NF-κB, cAMP responsive element binding protein (CREB), and AP-1. The inhibition of Akt has been found to prevent NF-κB activation by suppressing IKK phosphorylation and subsequent degradation of IκBα.

4.2. Phagocytosis

The prime function of monocytes and macrophages in innate host defense is known as phagocytosis, a process in which pathogen-infected or apoptotic cells are engulfed into a cytoplasmic compartment in receptor-mediated or non-receptor-mediated manners. Although phagocytic cells further contribute to the induction of inflammatory responses by producing various inflammatory cytokines and mediators, these cells function to remove inflammatory materials by up-regulating phagocytosis. Receptors known to regulate phagocytic function in macrophages include Fcγ receptors, such as FcγRI and FcγRIIIa, which are associated with the low molecular weight γ-subunit and bear immunoreceptor tyrosine-based activation motifs (ITAMs) (58). The phosphorylation of this motif plays a critical role in recruiting several signaling enzymes and adaptor molecules that are required for phagocytosis. One of the major enzymes that phosphorylates ITAMs is Src kinase. After activation of Src kinase, additional signaling enzymes, including PIP3-dependent signaling enzymes such as PI3K, PDK1, and Akt, form biochemically active complexes. Ganesan *et al.* (2004) found a novel role for Akt in Fcγ receptor-mediated phagocytosis (23). This study demonstrated that myristoylated Akt but not dominant-negative CAAX-Akt significantly increased phagocytosis by activating p70S6K (23). Rac has also been reported to act as an upstream regulator of p70S6K (59). Akt-mediated phagocytosis is thus thought to be regulated by various substrate proteins such as Rac and p70S6K. More interestingly, Akt1 plays a critical role in the modulation of intracellular growth of phagocytosed bacteria in macrophages (60). To this end, Neefjes *et al.* screened inhibitors of signaling enzymes for anti-bacterial activity against intracellular bacteria such as *Salmonella typhimurium*, and two inhibitors targeted to Akt1, H-89 and ETB067, were found to be the most promising drugs (60). The results of this study further demonstrate that Akt1 regulates intracellular survival of *Salmonella typhimurium* via SopB by modulating the actin cytoskeleton through PAK4 and modulating phagosome-lysosome function through the AS160-RAB14 pathway (60). Because the doses of ETB067 and its derivatives tested did not affect mouse viability or cause any detectable tissue damage, the authors of this study suggested that Akt1 could be targeted for development of novel antibiotics with no side effects (60). Akt has also been reported to play a critical role in controlling the apoptosis of macrophages that have phagocytosed bacteria such as *Salmonella typhimurium* (61). Similar to the general apoptotic pathway, infection with *S. typhimurium* stimulated a signaling cascade composed of Rho-GTPases, c-Jun N-terminal kinase (JNK), p38, Akt and Raf-1 (61).

4.3. Macrophage migration and chemotaxis

Migration of macrophages to the proximity of inflamed areas or tumors is an important immunobiological event. To clear apoptotic bodies, macrophages must interact with various pathogens or cancer cells; migration thus plays a critical role in the ability of macrophages to encounter their phagocytic targets. Macrophage migration is usually modulated by chemokines such as colony stimulating factor (CSF)-1, growth factors such as insulin-like growth factor (IGF-1), adhesion molecules such as LFA-1 and integrins, and surface proteoglycans such as osteoprotegerin (62). Stimulation of macrophages by chemokines and growth factors through chemokine receptors, a subfamily of G-protein-coupled receptors, results in the activation of a variety of intracellular signaling cascades composed of PI3K, PTEN, Rho A, PKCζ, LIM domain kinase (LIMK), syndecan, and cofilin (62-66). Although the exact molecular mechanisms driving macrophage chemotaxis are not clearly defined, several lines of evidence obtained using molecular biological and pharmacological inhibitory strategies suggest that Akt acts as an upstream signaling regulator of intracellular

Table 3. Natural occurring compounds inhibiting Akt activation in macrophages and monocytes

Compound	Cell	Action target of Akt	Ref
Desmethylanhydro-icaritin	Macrophages and endotoxemic mice	NF-κB-regulated inflammatory gene expression	(105)
Pimaradienoic acid	RAW 264.7 macrophages	Production of NO, PGE_2, and IL-6	(106)
6-Shogaol	Murine macrophages	Induction of iNOS and COX-2	(107)
Acacetin	Murine macrophages	Induction of iNOS and COX-2	(108)
C-Phycocyanin	Mouse macrophages	Expression of MDR1	(109)
Cryptotanshinone	RAW264.7	Chemotactic migration	(67)
Inotilone	Murine macrophage	Induction of iNOS and COX-2	(110)
Luteolin	RAW 264.7cells	Endotoxin-stimulated phosphorylation cascade and proinflammatory cytokine production	(111)
Miyabenol A	RAW 264.7 cells	NO Production	(112)
Pterostilbene	Murine macrophages	Expression of iNOS and COX-2	(113)
Rosmanol	RAW 264.7 macrophages	Induction of iNOS and COX-2	(114)
Lutein	RAW 264.7 macrophages and peritoneal macrophages	Induction of NF-κB-dependent gene expression	(115)
Resveratrol	RAW 264.7 macrophages	The expression of MCP-1 and Nox1	(116)

chemotactic processes. First, knocking down Akt2 expression resulted in a strong impairment in chemotaxis and migration of monocytic THP-1 cells and mouse peritoneal macrophages to CSF-1 and MCP-1 (64). Second, Akt2 siRNA strikingly reduced both actin polymerization and the phosphorylation of PKCζ and LIMK/cofilin, which are essential events for chemotaxis (64). Furthermore, cryptotanshinone, the major tanshinone isolated from *Salvia miltiorrhiza* Bungea, a strong anti-inflammatory drug, impaired chemotactic migration of macrophages to complement 5a (C5a) or macrophage inflammatory protein-1α by inhibiting PI3K (p110γ) membrane translocation and Akt phosphorylation (67). Similarly, osteoprotegerin-stimulated monocyte chemotaxis is associated with signaling events mediated by protein kinase C, PI3K/Akt, and tyrosine kinase via syndecan-1 (62).

4.4. LPS tolerance

Activation of macrophages by LPS triggers a negative feedback regulatory pathway involved in maintaining balanced inflammatory responses. The two major events that have been demonstrated to provide this negative feedback are regulation of TLR4 gene expression and activation of negative modulators including suppressor of cytokine signaling 1 (SOCS1) and interleukin-1 receptor-associated kinase-M (IRAK-M) (68, 69). Strong induction of these responses is linked to LPS tolerance, and defects in these responses promote hyperresponsiveness to LPS and consequently, suppress the development of endotoxin tolerance (68). Numerous studies have added to the understanding of the major signaling enzymes that mediate these negative regulatory responses. For example, activation of the PI3K/Akt pathway has been shown to block LPS-induced MAPK and NF-κB signaling cascades in monocytes and dendritic cells, resulting in diminished secretion of pro-inflammatory cytokines (70). More interestingly, Akt1-deficient macrophages demonstrate enhanced responsiveness to endotoxin, and Akt1-deficient mice do not exhibit LPS tolerance *in vivo* (71). Tsatsanis *et al.* found that the key molecules regulating this enhanced sensitivity to LPS are microRNAs (71). Indeed, expression levels of miR-155 and let-72 are remarkably different between wild type and Akt1-deficient mice, and these molecules have been demonstrated to modulate the expression levels of SOCS-1 and TLR4 (71). These findings suggest that Akt1 may modulate LPS sensitivity or tolerance by regulating miR-155 and let-72, resulting in subsequent regulation of TLR4 and SOCS1 expression.

5. DEVELOPMENT OF AKT INHIBITORS AS NEW IMMUNOSUPPRESSIVE DRUGS

5.1. Akt inhibitor development

Understanding the functional and biological significance of Akt in tumorigenesis has led to the development of novel Akt inhibitors. For several years, greatly increased numbers of chemical compounds have been designed and synthesized as novel inhibitors of Akt. Structural understanding of the functions of Akt has allowed the development of selective and strong inhibitors that are structurally optimized to bind target areas such as the ATP binding motif or other allosteric sites (72, 73). GSK690693, Se,Se'-1,4-phenylenebis(1,2-ethanediyl)bisisoselenourea, 4-(4-aminopiperidin-1-yl)-7H-pyrrolo[2,3-d]pyrimidines, orally bioavailable indazole-pyridine series, a series of N(1)-(5-(heterocyclyl)-thiazol-2-yl)-3-(4-trifluoromethylphenyl)-1,2-propanediamines, 5-methyl-4-phenyl-1H-pyrazole, tetrasubstituted pyridines, aminofurazans, 2,3,5-trisubstituted pyridines, and 2,3,5-trisubstituted pyridines are examples of recently developed Akt inhibitors with IC_{50} values ranging from 0.001 to 50 μM (74-77). In addition to direct assessment of enzymatic activity, the biological activities of these inhibitors have mostly been tested in cancer and cardiovascular disease settings; for example, studies have addressed the ability of Akt inhibitors to reduce hypotension and to arrest tumor growth in human breast carcinoma, colon cancer, and glioblastoma (74, 76). Because no trials have assessed the immunopharmacological efficacy of these inhibitors under inflammatory conditions *in vitro* and *in vivo*, systematic approaches could lead to the discovery of promising immunosuppressive or anti-inflammatory drugs that target the pivotal role of Akt during inflammatory responses.

5.2. Akt inhibitors that inhibit production of inflammatory mediators

Although few experimental trials of selective Akt inhibitors have been reported, a large amount of evidence suggest that naturally occurring anti-inflammatory agents and plant extracts act by suppressing the PI3K/Akt pathway (Table 3 and 4). For example, *Eleutherococcus senticosus* extract has been reported to attenuate LPS-

Table 4. Plant extracts inhibiting Akt activation in macrophages and monocytes

Plant	Cell	Target Akt pathway	Ref
Francisella tularensis	murine bone marrow macrophages	Suppression of cytokine production through the inhibition of NF-κB	(117)
Eleutherococcus senticosus	murine macrophages	Attenuation of LPS-induced iNOS expression by inhibiting the Akt and JNK pathways	(78)
Dichroa febrifuga Lour.	RAW 264.7 cells peritoneal macrophages macrophage	Inhibition of the production of IL-1β and IL-6, NF-κB activation, IκBα degradation, and IKK, Akt, ERK1/2 and JNK activities	(80)
Eleutherococcus senticosus	macrophage	Attenuation of LPS-induced iNOS expression through the inhibition of Akt and JNK pathways	(78)
Acer tegmentosum	RAW 264.7 cells	Suppression of the activation NF-κB, AP-1, and CREB, and upstream inflammatory signaling cascades, including Akt, p38, and JNK.	(79)
Inonotus obliquus	RAW 264.7 cells macrophage	the inhibition of NF-κB through the PI3K/Akt/IκB pathway and the inhibition of JNK activation	(118)

induced iNOS expression by inhibiting the Akt and JNK pathways in murine macrophages (78). It has been reported that treatment with a 70% ethanol extract of *Acer tegmentosum* results in a dose-dependent reduction in the production of NO, TNF-α, and PGE_2 in LPS-activated RAW264.7 cells and peritoneal macrophages through a transcriptional mechanism (79). *E. senticosus* extract was further shown to regulate the activation of NF-κB, AP-1, and CREB by effectively inhibiting upstream inflammatory signals, including Akt, p38, and JNK (79). Notably, treatment with an aqueous extract of *Dichroa febrifuga* was shown to result in a decrease in inflammatory mediator production in LPS-activated peritoneal macrophages by suppressing the activity of IKK/IκB/NF-κB and the phosphorylation of Akt, ERK1/2, and JNK (80). Furthermore, the inhibitory effects of tetramethylpyrazine on LPS-induced over-production of NO and iNOS in N9 microglial cells have also been found to be mediated by blocking MAPK and PI3K/Akt activation and suppressing ROS production (81). Cordycepin, which is derived from *Cordyceps militaris*, a traditional medicinal mushroom that parasitizes caterpillars, inhibits the production of NO by suppressing NF-κB activation and phosphorylation of Akt and p38, resulting in down-regulation of iNOS and COX-2 gene expression (82). Natural products and chemical compounds that inhibit Akt are summarized in Table 2.

5.3. Akt inhibitors with *in vivo* therapeutic effects in inflammatory models

Acute and chronic mouse and rat models have been widely used to investigate the efficacy of potential anti-inflammatory agents identified in *in vitro* screens. In these models, croton oil, carageenan, arachidonic acid, and collagen have been employed to induce inflammation (51, 83). Similar to the results of *in vitro* experiments, histamine, prostaglandins and some pro-inflammatory cytokines, such as TNF-α and IL-1β, were shown to be important in boosting inflammatory symptoms (51). Septic shock provides a representative model of acute inflammation triggered by Gram-negative bacteria-derived endotoxins like LPS that results in significant production of TNF-α and NO. In the ear edema model, inflammation is acutely induced by croton oil and arachidonic acid (84). Rat and mouse models of arthritis induced by adjuvant containing type II collagen or Mycobacteria are also used as representative chronic inflammatory models for drug efficacy tests (54).

Few studies have examined the relevance of Akt in the onset of septic shock or sepsis. The Akt inhibitory compound ferulaldehyde, a natural intermediate of polyphenol metabolism of intestinal microflora, enhances the survival rate of LPS-treated mice (85), and endotoxin-induced cardiac dysfunction and lethality are closely associated with the activity of PI3K and the Akt pathway (86), suggesting a potential role for Akt in acute inflammatory septic shock. Despite these findings, no reports on the therapeutic activity of known Akt inhibitors in septic shock have been published. Recently, however, the novel Akt inhibitor JS-III-049 was shown to protect mice against lethal responses to LPS/D-galactosamine (D-GalN) (28), suggesting that these Akt inhibitors may be considered a new class of therapeutic drugs to treat septic shock or endotoxemia.

Similarly, the pathological roles of Akt in rheumatoid arthritis have not been fully elucidated. It has been reported that PI3Kγ deficiency does not alter the recruitment of inflammatory cells, but significantly reduces cartilage damage through reduced expression of matrix metalloproteinases in fibroblasts and chondrocytes (87). Imatinib mesylate, a specific inhibitor of the PDGF receptor tyrosine kinase, the novel A_3AR agonist CF502, which has high affinity and selectivity for the human A_3AR, and the colchicine-derived compound CT20126 have been reported to mediate anti-arthritic activity by suppressing the Akt pathway (88). More interestingly, adenovirus-mediated PTEN gene transfer remarkably attenuated collagen-induced arthritis in rats and simultaneously suppressed the PI3K/Akt pathway in rheumatoid arthritis synovial tissue (89). Although these findings clearly implicate the Akt pathway as a therapeutic target for the treatment of rheumatoid arthritis, no trials of specific Akt inhibitors have been reported. Further evaluation of the therapeutic use of Akt inhibitors will provide an opportunity to begin to develop a novel class of anti-rheumatoid arthritis drugs.

5.4. Novel Akt binding site: implications for development of a novel class of anti-inflammatory Akt inhibitors

Although novel Akt inhibitors are often tested for their anti-cancer activity, few studies have examined the therapeutic activity of these newly developed inhibitors in various inflammatory diseases. Numerous lines of evidence indicating that Akt plays a critical role in the regulation of macrophage-mediated immune responses suggest that novel Akt inhibitors may also be useful in the treatment of a variety of immunological diseases. Indeed, our group

found that the dihydroxyl benzene metabolite hydroquinone and its derivatives demonstrated strong anti-inflammatory properties in the LPS/D-GalN-induced septic shock model by suppressing activation of NF-κB (28). Interestingly, these compounds were found to significantly inhibit Akt kinase activity by binding to the sulfhydryl group of C310 (28). That the sulfhydryl group of C310 serves as a novel allosteric site was demonstrated by the ability of hydroquinone derivatives to dramatically reduce the phosphorylation of T308 and S473 (28). Due to this pharmacological activity, the survival rate of mice undergoing endotoxin shock was remarkably enhanced by hydroquinone derivatives (28). Akt inhibitory compounds have also been demonstrated to strongly suppress the up-regulation of serum TNF-α, a major septic shock-causing pro-inflammatory cytokine that results from NF-κB activation, in response to LPS and D-GalN treatment (90, 91). These results suggest that in addition to the *in vivo* anti-inflammatory activities of Akt inhibitors that target C310, the biochemical and pharmacological significance of the C310 residue as a novel allosteric site could be considerable. The C310 reside was also proposed as a pharmacological target residue by the Yu group (92, 93). The Yu group explored the mechanisms driving the anti-cancer activity of natural antibiotics such as lactoquinomycin and demonstrated that similar to hydroquinone derivatives, these drugs function by targeting the Akt residue C310 (92). Derivatives of these antibiotics also exhibited Akt inhibitory and anti-cancer activities (93), confirming the critical function of residue C310 in the regulation of Akt activity. Indeed, it has been proposed that pyranonaphthoquinone lactones can be considered a new class of Akt-selective kinase inhibitors that are capable of alkylating a regulatory loop cysteine with IC_{50} values of 0.04 to 1.5 μM (93). Ongoing studies aim to prove the anti-inflammatory activities of these novel Akt inhibitors.

6. SUMMARY AND PERSPECTIVE

Numerous studies of the involvement of Akt in phagocytosis, intracellular bacterial infections, LPS tolerance, the production of inflammatory cytokine and mediators, and macrophage migration have revealed the important role of Akt in macrophage-mediated innate immunity. Importantly, a variety of inflammatory diseases such as rheumatoid arthritis, atherosclerosis, and osteoporosis are regulated by macrophage activation. The critical role of Akt in macrophage activation led us to consider that inhibiting Akt activity may be a useful therapeutic strategy in macrophage-mediated diseases. Recently, a few researchers have investigated the possibility that Akt inhibitors could be useful for this purpose. Considering that Akt-C310-targeted hydroquinone derivatives display strong anti-inflammatory and anti-septic shock activities, future studies will aim to develop these compounds as Akt-targeted immunomodulatory drugs. We expect that novel and safe Akt inhibitors with strong immunosuppressive and anti-inflammatory properties will be developed in the near future.

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Abbreviations: NO: nitric oxide, ROS: reactive oxygen species, TNF: tumor necrosis factor, IL: interleukin, iNOS: nitric oxide synthase, COX: cyclooxygenase, PRRs: pattern recognition receptors, TLR: Toll-like receptor, LPS: lipopolysaccharide, MPAKs: mitogen activated protein kinases, NF-κB: nuclear factor-κB, AP-1: activator protein-1, IKK: IκBα kinase, PGN: peptidoglycan, PH: pleckstrin homology, HM: hydrophobic motif, PIP3: phosphatidylinositol-(3,4,5)-triphosphate, IRF: interferon regulatory factor, JAK-2: Janus kinase-2, NIK: nuclear factor-κB-inducing kinase, PI3K: phosphoinositide 3-kinase, PDK1: phosphoinositide-dependent kinase 1, ERK: extracellular signal-regulated kinase, JNK: c-Jun N-terminal kinase, CREB: cAMP responsive element binding protein, ITAMs: immunoreceptor tyrosine-based activation motifs, CSF-1: colony stimulating factor-1, IGF-1: insulin-like growth factor-1, LIMK: LIM domain kinase, C5a: complement 5a, SOCS1: suppressor of cytokine signaling 1, IRAK-M: interleukin-1 receptor-associated kinase-M, D-GalN: D-galactosamine

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