Adenosine 5’-Monophosphate-Activated Protein Kinase Promotes Macrophage Polarization to an Anti-Inflammatory Functional Phenotype

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Herein, we demonstrate a role of AMP-activated protein kinase (AMPK) as a potent counterregulator of inflammatory signaling pathways in macrophages. Stimulation of macrophages with anti-inflammatory cytokines (i.e., IL-10 and TGFβ) resulted in the rapid phosphorylation/activation of AMPK, whereas stimulation of macrophages with a proinflammatory stimulus (LPS) resulted in AMPK dephosphorylation/inactivation. Inhibition of AMPKα expression by RNA interference dramatically increased the mRNA levels of LPS-induced TNF-α, IL-6, and cyclooxygenase-2. Likewise, expression of a dominant negative AMPKα1 in macrophages enhanced TNF-α and IL-6 protein synthesis in response to LPS stimulation, while diminishing the production of IL-10. In contrast, transfection of macrophages with a constitutively active form of AMPKα1 resulted in decreased LPS-induced TNF-α and IL-6 production, and heightened production of IL-10. In addition, we found that AMPK negatively regulated LPS-induced IκB-α degradation and positively regulated Akt activation, accompanied by inhibition of glycogen synthase kinase β and activation of CREB. Thus, AMPK directs signaling pathways in macrophages in a manner that suppresses proinflammatory responses and promotes macrophage polarization to an anti-inflammatory functional phenotype. The Journal of Immunology, 2008, 181: 8633–8641.

The AMP-activated protein kinase (AMPK)3 is an evolutionarily conserved serine/threonine kinase that regulates energy homeostasis and metabolic stress. When the cellular AMP/ATP ratio is high, AMPK is activated, switching off ATP-consuming anabolic pathways and switching on ATP-producing catabolic pathways (1). Mammalian AMPK is a heterotrimeric complex comprised of a catalytic α subunit and regulatory β and γ subunits. Each subunit has two or three isoforms (α1, α2, β1, β2, γ1, γ2, γ3) encoded by different genes (2). Phosphorylation of the threonine 172 residue of the α subunit is crucial for the AMPK activity (3). There are two kinases that have been established as upstream activators of AMPK: the protein kinase LKB1/STRAD/MO25 complex (4) and the calmodulin-dependent protein kinase kinase β (CAMKKβ) (5). Although the LKB1 complex phosphorylates AMPK in response to changes in the AMP/ATP ratio (6), CAMKKβ phosphorylates AMPK in response to an increase in intracellular Ca2+ level (5). In liver, active AMPK inhibits fatty acid synthesis and cholesterol synthesis (7) while in heart and skeletal muscle it stimulates fatty acid oxidation and glycolysis (8, 9). AMPK has also been shown to inactivate the mammalian target of rapamycin (mTOR) pathway via phosphorylation and activation of the mTOR inhibitor, tuberous sclerosis complex-2 (TSC2) (10). This would typically occur when AMPK is activated as a result of energy deprivation, the net result being suppression of protein synthesis and cell growth. Studies demonstrating the role of AMPK in improvement of insulin sensitivity and glucose homeostasis have identified AMPK as a target for the treatment of type 2 diabetes and obesity (11).

A potential role of AMPK in suppression of inflammatory responses has been suggested by studies using the pharmacological activator of AMPK, 5-aminoimidazole-4-carboxamide ribose (AICAR). For example, treatment of mice with AICAR was found to reduce the severity of experimental autoimmune encephalomyelitis (12). AICAR has also been shown to reduce inducible NO synthase activity by adipocytes, macrophages, myocytes, and glial cells (13, 14). However, AICAR is taken up by cells and converted to AMP analog 5-aminoimidazole-4-carboxamide-1-β-d-ribofuranoside, which mimics the effect of AMP, thus AICAR is a nonspecific activator of AMPK and has the potential to activate other AMP-sensitive enzymes (15). Indeed, there are recent reports in which the anti-inflammatory effects of AICAR were determined to be independent of AMPK activity, thus clouding the interpretation of studies using this reagent (16, 17). As part of our ongoing studies of the signaling pathways that govern macrophage behavior, we investigated the role of AMPK in the regulation of macrophage inflammatory activity in response to physiological stimuli. We demonstrate that AMPK in macrophages can be rapidly activated or inactivated by anti-inflammatory or proinflammatory stimuli, respectively, and we provide evidence that AMPK acts as a central regulator of macrophage inflammatory function.
Materials and Methods

Reagents

LPS (Escherichia coli serotype O111:B4) was purchased from Sigma-Aldrich. Mouse rIL-10 and human rTGFβ were purchased from R&D Systems. Western blot detection of specific proteins used the following primary Abs: anti-phospho-AMPKα (Thr172), anti-AMPK-α, anti-phospho-tyrosyl-CoA carboxylase (ACC) (Ser79), anti-ACC, anti-phospho-GSK-3β (Ser9), anti-GSK-3β, anti-phospho-Akt (Ser473), anti-Akt, anti-phospho-CREB (Ser133), anti-CREB, anti-Ibα (Cell Signaling Technology), anti-AMPKα1, anti-AMPKα2 (Abcam), anti-β-actin (Sigma-Aldrich), and HRP-conjugated secondary Ab (Jackson ImmunoResearch Laboratories).

Western blot analysis

Murine bone marrow-derived macrophages and human monocyte-derived macrophages were generated as previously described (18, 19). Macrophages were lysed in a lysis buffer containing 125 mM Tris (pH 6.8), 2% SDS, 20% glycerol, 200 μM PMSF, protease inhibitor mixture (Promega), and phosphatase inhibitor mixture (Pierce). Total protein content of the samples was assessed by BCA protein assay (Pierce). Equal amounts of protein were separated on 10% Criterion gels (Bio-Rad) by SDS-PAGE. Proteins were transferred to nitrocellulose membranes (Hybond; Amersham Biosciences) using a Trans-Blot SD semidry electrophoretic transfer system. Western blot analysis system (Amersham Biosciences), and the membranes were exposed to Kodak Biomax XL X-ray film (Eastman). Densitometric analysis was performed using the Bio-Rad Quantity One 8 software associated with Bio-Rad Fluor-S MultiImager and FX phosphorimager systems.

ELISA

Following stimulation in 96-well plates, supernatants were collected and assayed by ELISA using OptEIA sets (BD Biosciences Pharmingen) according to the manufacturer’s instructions. Analysis was performed using an E-max precision micro plate reader (Molecular Devices).

RNA interference

Murine bone marrow-derived macrophages were transfected with 0.5 μg AMPK α1/2 small interfering RNA (siRNA) or nonspecific control siRNA (Dharmacon) using Amaxa Biosystem’s nucleofection technology according to the manufacturer’s instructions. Following nucleofection, the macrophages were plated in 12-well plates in RPMI 1640 (HyClone) medium containing 20% FBS (Atlanta Biologicals), 100 mM HEPES, 50 μg/ml gentamicin, 0.5 mM l-glutamine, and 1.5 mM GlutaMAX (Invitrogen). The cells were analyzed 72 hr post-transfection.

Real-time RT-PCR analysis

mMACs One-step cDNA Kits (Miltenyi Biotec) were used for RNA isolation and cDNA synthesis. cDNAs were amplified in a 20 μl reaction volume containing SYBR Green (New England Biolabs) and analyzed using a DNA Opticon 2 Monitor (Bio-Rad). IL-6, TNF-α, and cyclooxygenase-2 (COX-2) expression was analyzed by Quantitect Primer Assays (Qiagen). cDNA concentrations in each sample were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S rRNA. cDNA concentrations in each sample were normalized using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 18S rRNA. The relative expression software tool was used to quantify mRNA expression of each gene (20).

Generation of stable transfectants

Dominant negative (DN-AMPKα1) and constitutively active (CA-AMPKα1) forms of AMPK were generated in the Carling laboratory as described previously (21). DN-AMPKα1 and CA-AMPKα1 coding regions were sub-cloned into pcDNA-Zeo (Invitrogen) and 2 μg empty pcDNA-Zeo-AMPKα1 were transfected into the B6J2 macrophage cell line (22) by using nucleofection technology (Amaxa Biosystems) according to the manufacturer’s instructions. Selection of the transfectants was achieved via addition of zeocin to the cultures.

Statistical analysis

Statistical significance between groups was calculated with an unpaired Student’s t test, with a value of p < 0.05 considered statistically significant.


FIGURE 2. AMPK activity is rapidly modulated by anti-inflammatory and proinflammatory stimuli. A and B, Anti-inflammatory cytokines IL-10 and TGFβ enhance the levels of phosphorylated AMPK in mouse macrophages. Bone marrow-derived macrophages were stimulated with 20 ng/ml IL-10 or 5 ng/ml TGFβ for the time points indicated. C, LPS stimulation diminishes AMPK phosphorylation in mouse macrophages. Bone marrow-derived macrophages were stimulated with 100 ng/ml LPS for the time points indicated. D, LPS stimulation decreases AMPK phosphorylation in human macrophages. Human macrophages were stimulated with 100 ng/ml LPS for the time points indicated. Western blot was performed using Abs against phospho-AMPK catalytic domain, which is an indication of AMPK activity. Bars show the ratio of phosphorylated to total AMPK protein as assessed by densitometry and shown as bar graphs. Data shown are representative of four independent experiments with similar results.

Results

AMPKα1 is the predominant AMPKα isoform expressed by macrophages

Expression of AMPKα isoforms by murine bone marrow-derived macrophages, the murine macrophage cell line B6J2, and human monocyte-derived macrophages was evaluated by Western blot and real-time RT-PCR. As shown in Fig. 1A, Western blot analysis revealed expression of the 62 kDa AMPKα1 protein in each of the macrophage samples tested, whereas no detectable expression of AMPKα2 protein was observed. Analysis of AMPKα mRNA expression by murine bone marrow-derived macrophages (Fig. 1B) did show detectable levels of AMPKα2; however, AMPKα1 was expressed at a 19-fold higher level. Likewise, AMPKα1 was expressed at a 39-fold higher level in murine macrophage cell line B6J2 (Fig. 1C). In human monocyte-derived macrophages AMPKα2 expression level was negligible (8200-fold less than AMPKα1) (Fig. 1D). These data indicated that AMPKα1 is the dominant AMPKα isoform expressed in macrophages, and our subsequent investigation focused on the manipulation of AMPKα1 expression and activity as a means to elucidate the role of AMPK in macrophages.

AMPK activity is rapidly modulated by anti-inflammatory and proinflammatory stimuli

We evaluated the effect of anti-inflammatory and proinflammatory stimuli on the phosphorylation of the Thr-172 residue of the AMPKα catalytic domain, which is an indication of AMPK activation. Bone marrow-derived macrophages were stimulated with the typically anti-inflammatory cytokines IL-10 and TGFβ or with the proinflammatory TLR4 agonist LPS. Stimulation of bone marrow-derived macrophages with IL-10 (Fig. 2A) and TGFβ (Fig. 2B) resulted in a rapid and marked increase in the phosphorylation level of AMPK, evident at 10 min poststimulation, which was sustained over an 18-h time period. In contrast, LPS stimulation resulted in a significant reduction of AMPK phosphorylation, which was maintained through a 1-h time point, with a return to the phosphorylated state by the 6-h time point tested (Fig. 2C). We hypothesize that this return of AMPK phosphorylation may be due to an autocrine response to IL-10 produced by macrophages in response to LPS stimulation. LPS stimulation also resulted in diminished AMPK phosphorylation in human macrophages over a 30-min time period, which returns to basal levels at the 1-h time point (Fig. 2D). Interestingly, a second reduction in AMPK phosphorylation is apparent at 6 h, which may be due to an autocrine influence of proinflammatory cytokine production. These data indicate that anti-inflammatory stimulus increases, while proinflammatory stimulus decreases, AMPK activation in macrophages.

Inhibition of AMPK expression or activity elevates LPS-induced macrophage inflammatory function

AMPK function in macrophages was evaluated by inhibition of AMPK expression and activity with use of siRNA silencing and a DN-AMPKα1 mutant. Bone marrow-derived macrophages were transfected with AMPKα1/α2 siRNA or a scrambled control siRNA. Following transfection, LPS-induced TNF-α, IL-6, and COX-2 mRNA was evaluated. As shown in the Western blot analysis depicted in Fig. 3A, macrophages transfected with AMPKα siRNA displayed a substantial reduction in AMPKα protein as compared with control siRNA transfected or untransfected macrophages. Typically, including in the experiment shown in Fig. 3A, siRNA suppression of AMPKα achieved an approximate 70% reduction in protein as assessed by densitometry of Western blot analyses. Suppression of the AMPKα catalytic domain expression in macrophages resulted in a dramatic increase in TNF-α (4-fold), IL-6 (3-fold), and COX-2 (10-fold) mRNA expression in response to LPS stimulation as compared with control siRNA-treated macrophages (Fig. 3, B–D). Similar results were obtained with
blockade of AMPKα activity. In these experiments, the B6J2 macrophage cell line was stably transfected with DN-AMPKα1. Empty vector transfected macrophages (pcDNA-Zeo) were used as control. To demonstrate that the macrophages expressing DN-AMPKα1 have impaired AMPK activity, phosphorylation (Ser 79) status of the AMPK substrate ACC was evaluated by Western blot. As shown in Fig. 4A, DN-AMPKα1 macrophages display a 50% reduction in basal level of ACC Ser79 phosphorylation and, therefore, impaired AMPK activity, as compared with control cells. LPS-induced TNF-α, IL-6, and IL-10 production of DN-AMPKα1 macrophages was analyzed by ELISA (Fig. 4, B–D). The B6J2 cell line expressing DN-AMPKα1 produced significantly more proinflammatory TNF-α and IL-6, whereas production of the anti-inflammatory IL-10 was significantly reduced, as compared with the control cell line (pcDNA-Zeo empty vector transfectants).

Expression of a constitutively active AMPKα1 results in reduced macrophage inflammatory cytokine production and enhanced production of IL-10

We next evaluated the impact of elevated AMPK activity on macrophage inflammatory activity with use of CA-AMPKα1. Stable transfectants of B6J2-expressing CA-AMPKα1 were generated and constitutive AMPK activation in these cells was confirmed by evaluation of the Ser79 phosphorylation of the AMPK substrate ACC by Western blot. CA-AMPKα1 macrophages displayed 3.5-fold increased basal level of ACC Ser79 phosphorylation and, therefore, AMPK activity, as compared with empty vector transfected macrophages (Fig. 4A). Cytokine production by CA-AMPKα1 macrophages in response to LPS stimulation was evaluated by ELISA (Fig. 4, E–G). B6J2 macrophages transfected with CA-AMPKα1 displayed significantly lower levels of LPS-induced TNF-α and IL-6 production as compared with the control cell line (pcDNA-Zeo transfectants), whereas IL-10 production was significantly higher in the CA-AMPKα1-expressing cells as compared with controls (Fig. 4, E–G). These data provide further evidence that AMPK counterregulates the inflammatory function of macrophages.

Evidence for AMPKα1 regulation of inhibitory κB kinase (IKK)/NF-κB, Akt-, GSK3-β-, and CREB-mediated signaling pathways

We considered a number of downstream targets of AMPK as possible mediators of the counterinflammatory activity we observed. For example, in endothelial cells AMPK activity has been implicated as a negative regulator of the transcription factor NF-κB (23, 24), which is well-established as playing a critical role in the induction of proinflammatory gene expression (25). In unstimulated cells, the NF-κB p65/p50 heterodimer is held inactive in the cytoplasm by the inhibitory protein IκB. Proinflammatory stimuli activate IKK, which in turn phosphorylates IκB, resulting in its ubiquitination-mediated degradation, allowing liberated NF-κB to enter the nucleus and activate gene expression (25). Thus, degradation of IκB is widely used as an indication of NF-κB activation. We evaluated the levels of LPS-induced IκB degradation in macrophages expressing either DN-AMPKα1 or CA-AMPKα1. As shown in Fig. 5A, macrophages containing DN-AMPKα1 have increased IκB degradation after LPS stimulation as compared with empty vector-transfected macrophages. IκB is nearly absent at 10 min in macrophages expressing DN-AMPKα1 and remains low through the 30-min time point, whereas in the control cells maximum degradation occurs at 30 min, and the overall levels of IκBα protein are substantially higher than in the cells expressing DN-AMPKα1. In contrast, expression of CA-AMPKα1 both delays

FIGURE 3. Suppression of AMPK expression by RNA interference increases LPS-induced proinflammatory activity of macrophages. A, Bone marrow-derived macrophages were transfected with AMPKα1α2 siRNA or control siRNA or left untransfected. Then, 72 h post-transfection, the cells were lysed and Western blot was performed with Abs against AMPKα and β-actin. Data shown are representative of three independent experiments with similar results. B–D, Then, 72 h post-siRNA transfection, macrophages were stimulated with 100 ng/ml LPS for 4 (TNF-α and IL-6) or 2 h (COX-2) or left unstimulated. Real-time RT-PCR was performed for analysis of TNF-α, IL-6, and COX-2 mRNA expression. Data shown are mean ± SD of triplicate determinations (*, p < 0.05; **, p < 0.01) and representative of three independent experiments with similar results.
and decreases LPS-induced IκB degradation (Fig. 5B). These results suggest that AMPK acts as a negative regulator of the IKK/IκB/NF-κB pathway in macrophages.

AMPK has also been shown to modulate downstream events mediated by PI3K (26). PI3K indirectly activates the serine/threonine kinase Akt, which in turn can phosphorylate GSK3-β at Ser9, resulting in GSK3-β inhibition. The PI3K/Akt pathway has been shown to regulate the production of inflammatory cytokines in monocytes and macrophages (27), and inhibition of GSK3-β has been shown to inhibit TLR-mediated production of proinflammatory cytokines, while enhancing IL-10 production by human monocytes (27, 28). As shown in Fig. 5C, macrophages expressing DN-AMPKα1 display greatly reduced Akt Ser473 phosphorylation after LPS stimulation as compared with control, empty vector

FIGURE 4. Expression of DN-AMPKα1 or CA-AMPKα1 modulates the inflammatory response of macrophages. The B6J2 macrophage cell line was stably transfected with DN-AMPKα1 (A–D) or CA-AMPKα1 (A, and E–G). Empty vector (pcDNA-Zeo) B6J2 transfectants served as a control. Lysates of transfected macrophages were analyzed by Western blot using Abs against p-ACC and ACC. The p-ACC/total ACC ratio was analyzed by densitometry and shown as a bar histogram. Data shown are representative of two independent experiments with similar results (A). Transfected macrophages were stimulated with LPS at the concentrations shown for 18 h or left unstimulated. TNF-α, IL-6, and IL-10 levels were detected by ELISA. Data shown are mean ± SD of triplicate determinations (*, p < 0.05; **, p < 0.01) and representative of three independent experiments with similar results.
FIGURE 5. Expression of DN-AMPKα1 or CA-AMPKα1 modulates LPS induced iκB-α, Akt, GSK3-β, and CREB activity. A, B6J2 macrophages stably transfected with DN-AMPKα1 or empty vector were stimulated with 100 ng/ml LPS for the time points indicated. After cell lysis, Western blot was performed using an anti-iκB-α Ab. Bands were analyzed by densitometry, displayed as a bar histogram. B, B6J2 macrophages stably transfected with CA-AMPKα1 or empty vector were treated and analyzed as in A. C, Western blot was performed with Abs against p-Akt (Ser473) and total Akt. The p-Akt/total Akt ratio was analyzed by densitometry and shown as a bar histogram. D, B6J2 macrophages stably transfected with CA-AMPKα1 were stimulated and assayed as in C. E, DN-AMPKα1 transfectants were stimulated as in A and Western blot was performed with Abs against p-GSK3-β (Ser9) and total GSK3-β. The p-GSK3-β/total GSK3-β ratio was analyzed by densitometry and shown as a bar histogram. F, CA-AMPKα1 transfectants were stimulated and assayed as in E. G, DN-AMPKα1 transfectants were stimulated as in A and Western blot was performed with Abs against p-CREB (Ser133) and total CREB. The p-CREB/total CREB ratio was analyzed by densitometry and shown as a bar histogram. H, CA-AMPKα1 transfectants were stimulated and assayed as in G. Data shown are representative of two (B, D, E, and G) and three (A, C, F, and H) independent experiments with similar results.
Our data demonstrate an association of AMPK activity with a putative regulator of macrophage inflammatory function and promoter of behavior. The observed AMPK activity associated with macrophage functional polarization may suggest potential therapeutic target (40, 42). Thus, elucidation of the signal transduction pathways that can be manipulated has drawn attention to macrophages as a potentially modifiable cell type. The observation that macrophage functional phenotypes and their tissue environment (39–41) can produce different arrays of pro- and anti-inflammatory states, in most cases, the patterns of gene expression do not precisely fit the published patterns associated with these M1/M2 designations (36–38). Indeed, the array of possible functional outcomes of macrophage activation is quite broad (32), and we and others have demonstrated that macrophages can rapidly change their functional profile, both in vitro and in vivo, in response to changes in the microenvironment (39–41). Therefore, the M1/M2 nomenclature defines only the most polarized macrophage functional profiles among a wide array, and these polarized states are mutable. The observation that macrophage functional phenotypes can be manipulated has drawn attention to macrophages as a potential therapeutic target (40, 42). Thus, elucidation of the signaling pathways that regulate macrophage functional polarization will aid in the design of strategies for modification of macrophage behavior.

In the present study, we have identified AMPK as a potent counterregulator of macrophage inflammatory function and promoter of macrophage polarization toward an anti-inflammatory phenotype. Our data demonstrate an association of AMPK activity with reduced IκB degradation, enhanced Akt activity, GSK3-β inhibition, and activation of CREB. The enhanced Akt activation associated with AMPK activity is of interest given the mixed reports of the relationship of AMPK and Akt. Although some studies demonstrate a positive correlation of AMPK activity with that of Akt, as we report here, there are many reports of an association of AMPK activation with decreased Akt activation (reviewed in Ref. 26). We found that macrophages expressing a CA-AMPKα1 displayed enhanced Akt Ser473 phosphorylation as compared with empty vector transfected macrophages, whereas macrophages expressing a DN-AMPKα1 displayed diminished Akt Ser473 phosphorylation following LPS stimulation (Fig. 5E). It has been established that activation of Akt requires both phosphorylation of Thr308 by the phosphoinositide-dependent kinase 1 (43, 44) and phosphorylation of the Ser473 by the mTOR complex (mTORC2) (45). The serine/threonine kinase mTOR is a key regulator of the protein synthesis and cell growth. Due to energy depletion, AMPK inhibits mTORC1 by phosphorylating and activating the negative regulator of mTOR, TSC2 (10). In contrast to AMPK, Akt can activate mTORC1 by phosphorylating and inhibiting TSC2 (46, 47). mTOR is the only critical subunit shared by mTORC1 and mTORC2, and competition for mTOR interaction can occur between the mTORC1 and mTORC2 complexes (48). In macrophages expressing CA-AMPKα1, it is possible that inhibition of mTORC1 by AMPK could increase availability of mTOR to the mTORC2 complex. The enhanced phosphorylation of Akt in these cells could, therefore, be due to the ability of mTORC2 to phosphorylate Akt at Ser473. Akt would then be available to activate mTORC1 to balance mTORC1 activity in the cell.

The increased LPS-mediated Akt activation and GSK3-β inhibition in macrophages expressing CA-AMPKα1 indicates that active AMPK may exert its anti-inflammatory effects through an Akt/GSK3-β pathway (28). Interestingly, although we see an increase in LPS-mediated GSK3-β phosphorylation/inhibition in CA-AMPKα1-expressing macrophages, we do not observe any decrease in GSK3-β phosphorylation in macrophages expressing DN-AMPKα1 (Fig. 5E). This observation may be also explained by the influence of AMPK on the mTOR pathway. mTORC1 controls mRNA translation, in part, by phosphorylating and activating S6K1 which phosphorylates the S6 protein of the 40S ribosomal protein (49). S6K1 has been found to phosphorylate GSK3-β at Ser9, resulting in GSK3-β inactivation under conditions where mTORC1 is overactive (50). Therefore, it is likely that the absence of a decrease in GSK3-β Ser9 phosphorylation in DN-AMPKα1-expressing macrophages may be due to the overactivation of mTORC1, resulting from decreased AMPK activity. This would lead to enhanced S6K1 activity and, therefore, the maintenance of GSK3-β phosphorylation.

Since GSK3-β is a negative regulator of the transcription factor CREB, GSK3-β inactivation enhances CREB activity (28, 29). It is hypothesized that GSK3-β inhibition allows CREB to compete for the nuclear coactivator protein CREB-binding protein, also required for NF-κB function. This results in reduced NF-κB activation of proinflammatory gene expression and enhanced expression of CREB-activated IL-10 synthesis (28). This scenario fits well with our data, as we observe elevated CREB activation in macrophages expressing CA-AMPKα1 (Fig. 5H) as well as elevated IL-10 production (Fig. 4G). In earlier published work, GSK3-β inhibition was shown to decrease the binding of NF-κB p65 with CREB-binding protein but did not affect the levels of nuclear NF-κB p65 (28). In contrast, our finding that IκBα degradation is attenuated in CA-AMPKα1 transfectants (Fig. 5B) suggests that NF-κB activation and translocation to the nucleus are likely to be reduced in the presence of high AMPK activity.
In addition to IL-10 and TGFβ, we have found that other anti-inflammatory mediators such as IL-4, the PPARγ agonists 15dPJG2 and ciglitazone, as well as the green tea polyphenol epigallocatechin-3-gallate activate AMPK in macrophages (unpublished data), suggesting that, regardless of the initial signaling events involved, anti-inflammatory signals converge on AMPK as a central regulator that promotes progression to an anti-inflammatory phenotype. As mentioned previously, the two known upstream activators of AMPK are the kinases LKB1 (4) and CAMKKβ (5); however, a potential role of these kinases as activators of AMPK activation in macrophages has not been established. Due to the diversity of molecules capable of AMPK activation in macrophages, the identity of upstream activators is of particular interest and is under investigation in our laboratory.

AMPK has frequently been referred to as the “metabolic master switch” in discussion of its role in the regulation of energy homeostasis (51). The ability of AMPK to be rapidly activated by proinflammatory mediators such as IL-4, the PPARγ activators upregulated by N-α-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside is independent of AMP-activated protein kinase. J. Cell Biochem. 103: 931–940.

Mukundan, L., G. A. Bishop, K. Z. Head, L. Zhang, M. Wahl, and J. Suttles. 2005. TNF receptor-associated factor 6 is an essential mediator of CD40-activated proinflammatory pathways in monocytes and macrophages. J. Immunol. 174: 1081–1090.

Reynolds, M. J., Q. Liu, K. C. Brittingham, Y. Liu, M. Gruenenthal, C. Z. Gorgun, G. S. Hotamisligil, R. D. Stout, and J. Suttles. 2007. Deficiency of fatty acid-binding proteins in macrophages protects from development of experimental autoimmune encephalomyelitis. J. Immunol. 179: 313–321.

Palli, M. W., G. W. Horgan, and L. Demplle. 2002. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res. 30: e39.

Woods, A., D. Azzott-Marniche, M. Foret, S. C. Stein, P. Lenardch, P. Ferre, F. Foufelle, and D. Carling. 2000. Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. Mol. Cell. Biol. 20: 6704–6711.

Clemons-Miller, A. R., G. W. Cox, J. Suttles, and R. D. Stout. 2000. LPS stimulation of TNF-receptor deficient macrophages: a differential role for TNF-α autocrine signaling in the production of cytokine and nitric oxide production. Immunobiology 202: 477–492.

Okayasu, T., A. Tomizawa, K. Suzuki, K. Manaka, and Y. Hattori. 2008. PPARα activators upregulate eNOS activity and inhibit cytokine-induced NF-κB activation in an AMP-activated protein kinase-dependent manner. J. Biol. Chem. 283: 884–891.

Hattori, Y., Y. Nakano, A. Tomizawa, K. Inukai, and K. Kasai. 2008. High molecular weight adiponectin activates AMPK and suppresses cytokine-induced NF-κB activation in vascular endothelial cells. FEBS Lett.

Karn, M., and M. Delhase. 2000. The 1sβ kinase (IKK) and NF-κB: key elements of proinflammatory signaling. Semin. Immunol. 12: 85–98.

Motoshima, H., B. J. Goldstein, M. Igata, and E. Araki. 2006. AMPK and cell proliferation–AMPK as a therapeutic target for atherosclerosis and cancer. J. Physiol. 574: 63–71.

Guba, M., and N. Mackaman. 2002. The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytes. J. Biol. Chem. 277: 32124–32132.

Marin, M., K. Rehani, R. S. Jope, and S. M. Michalek. 2005. Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. Nat. Immunol. 6: 777–784.

Grimes, C. A., and R. S. Jope. 2001. CREB DNA binding activity is inhibited by glycogen synthase kinase-3 β and facilitated by lithium. J. Neurochem. 78: 2129–2132.

Platzer, C., E. Fritsch, T. Elsner, M. H. Lehmann, H. D. Volk, and S. Prosch. 1999. Cyclic adenosine monophosphate-responsive elements are involved in the transcriptional activation of the human IL-10 gene in monocytic cells. Eur. J. Immunol. 29: 3098–3104.

Thomson, D. M., S. T. Herway, N. Fillmore, H. Kim, J. D. Brown, J. R. Barrow, and W. W. Winder. 2008. AMP-activated protein kinase mediates phosphorylation transcriptions factors of the CREB family. J. Appl. Physiol. 104: 429–438.

Stout, R. D., and J. Suttles. 2007. Functional plasticity of macrophages: reversible adaptation to changing microenvironments. J. Leukoc. Biol. 76: 509–513.

Gordon, S. 2003. Alternative activation of macrophages. Nat. Rev. Immunol. 3: 23–35.

Mills, C. D., K. Kincaid, J. M. Alt, M. J. Heilmann, and A. M. Hell. 2000. M1/M2 macrophages and the Th1/Th2 paradigm. J. Immunol. 164: 6166–6173.

Mantovani, A., S. Sica, and M. Locati. 2005. Macrophage polarization comes of age. Immunity 23: 344–346.

Anderson, C. F., and D. M. Mosser. 2002. A novel phenotype for an activated macrophage: the type 2 activated macrophage. J. Leukoc. Biol. 72: 101–106.

Siáron, M., F. O. Martinez, D. A’Ambrosio, M.Gattorno, N. Polentarutti, M. Locati, A. Gregorio, A. Iemem, M. A. Cascallana, D. V. et al. 2006. Differential regulation of chemokine production by Fcε receptor engagement in human monocytes: association of CCL1 with a distinct form of M2 macrophage activation (M2B, Type 2). J. Leukoc. Biol. 80: 342–349.

Umemura, N., M. Saio, T. Suwa, Y. Kito, J. Bai, K. Konaka, G. F. Ouyang, M. Okada, M. Balazs, R. Adany, et al. 2008. Tumor-infiltrating myeloid-derived suppressor cells are pleiotropic-inflamed monocytes/macrophages that bear M1-like characteristics and exhibit M2b-like polarization. J. Immunol. 164: 1135–1144.

Stout, R. D., C. Jiang, B. Matta, I. Tietzel, S. K. Watkins, and J. Suttles. 2005. Macrophages sequentially change their functional phenotype in response to PGE2 and tumor-infiltrating lymphocytes. J. Immunol. 175: 342–349.

Watkins, S. K., N. K. Egilmez, J. Suttles, and R. D. Stout. 2007. IL-12 rapidly alters the functional profile of tumor-associated and tumor-infiltrating macrophages in vitro and in vivo. J. Immunol. 178: 1357–1362.

Grozinger, A., J. Kishihara, K. Koth, I. Muller-Molinet, S. Kannookadan, J. Uitakil, and S. Goedert. 2006. Mδ1 and Mδ2 can be re-polarized by Th2 or Th1 cytokines, respectively, and respond to exogenous danger signals. Immunobiology 211: 473–486.

Sica, A., P. Larghi, A. Mancino, L. Rehno, C. Poftta, M. G. Totaro, M. Rimoldi, S. K. Biowas, P. Allavena, and A. Mantovani. 2008. Macrophage polarization in tumour progression. Semin. Cancer Biol. 18: 349–355.
43. Alessi, D. R., M. Deak, A. Casamayor, F. B. Caudwell, N. Morrice, D. G. Norman, P. Gaffney, C. B. Reese, C. N. MacDougall, D. Harbison, et al. 1997. 3-Phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the Drosophila DSTPK61 kinase. Curr. Biol. 7: 776–789.

44. Stephens, L., K. Anderson, D. Stokoe, H. Erdjument-Bromage, G. F. Painter, A. B. Holmes, P. R. Gaffney, C. B. Reese, F. McCormick, P. Tempst, et al. 1998. Protein kinase B kinases that mediate phosphatidylinositol 3,4,5-trisphosphate-dependent activation of protein kinase B. Science 279: 710–714.

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

46. Inoki, K., Y. Li, T. Zhu, J. Wu, and K. L. Guan. 2002. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat. Cell Biol. 4: 648–657.

47. Manning, B. D., A. R. Tee, M. N. Logsdon, J. Blenis, and L. C. Cantley. 2002. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. Mol. Cell. 10: 151–162.

48. Bhaskar, P. T., and N. Hay. 2007. The two TORCs and Akt. Dev. Cell. 12: 487–502.

49. Tee, A. R., D. C. Fingar, B. D. Manning, D. J. Kwiatkowski, L. C. Cantley, and J. Blenis. 2002. Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. Proc. Natl. Acad. Sci. USA 99: 13571–13576.

50. Zhang, H. H., A. I. Lipovsky, C. C. Dibble, M. Sahin, and B. D. Manning. 2006. S6K1 regulates GSK3 under conditions of mTOR-dependent feedback inhibition of Akt. Mol. Cell. 24: 185–197.

51. Winder, W. W., and D. G. Hardie. 1999. AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am. J. Physiol. 277: E1–E10.