AICAR inhibits PPARγ during monocyte differentiation to attenuate inflammatory responses to atherogenic lipids

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Aims

Transcriptional regulation through peroxisome proliferator-activated receptor γ (PPARγ) is critical for an altered lipid metabolism during monocyte to macrophage differentiation. Here, we investigated how 5-aminoimidazole-4-carboxamide riboside (AICAR), an activator of AMP-dependent protein kinase (AMPK), affects PPARγ during monocyte differentiation.

Methods and results

During the differentiation of THP-1 monocytic cells or primary human monocytes to macrophages, we observed that AICAR inhibited the expression of PPARγ target genes, such as fatty acid-binding protein 4 or CD36. This effect was independent of AICAR conversion to AICAR ribotide and AMPK activation. While AICAR increased PPARγ mRNA expression that paralleled differentiation, it inhibited PPARγ protein synthesis without affecting PPARγ protein stability. Monocytes differentiated to macrophages in the presence of AICAR revealed an attenuated uptake of oxidized low-density lipoprotein (oxLDL) and reduced oxLDL-triggered c-Jun N-terminal kinase (JNK) activation. JNK and endoplasmic reticulum stress responses to the saturated fatty acid palmitate were attenuated as well, an effect mimicked by the knockdown of PPARγ. Although PPARγ has been reported to support alternative macrophage activation, AICAR did not inhibit interleukin-4-induced gene expression in differentiating monocytes.

Conclusion

Inhibition of PPARγ-dependent gene expression during monocyte differentiation may contribute to an AICAR-elicted macrophage phenotype characterized by reduced inflammatory responses to modified lipoproteins and saturated fatty acids.

Keywords

Fatty acids • Lipoproteins • Macrophages • Metabolism • Oxidized lipids

1. Introduction

Cells of the innate immune system play important roles in the pathogenesis of metabolic disorders. During atherosclerosis monocytes are recruited to the vessel wall, where they differentiate into macrophages, take up lipids and form foam cells.1 Similarly, in adipose tissue monocytes are recruited and differentiate to pro-inflammatory (M1) macrophages to drive obesity-associated insulin resistance.2 Strategies to prevent monocyte recruitment or their pro-inflammatory polarization attenuate the development of atherogenesis and insulin resistance. The process of monocyte to macrophage differentiation during metabolic disease is less understood. Differentiation involves changes in gene expression driven by multiple transcription factors. Among these, transcription factors of the peroxisome proliferator-activated receptor (PPAR) family are of particular importance due to their roles in regulating lipid metabolism and inflammation.3,4 The PPAR family consists of three ligand-activated nuclear receptors (PPARα, -γ, and -δ). Numerous studies suggested a beneficial role of PPAR activation during metabolic disease. Thus, synthetic PPAR agonists prevented the development of atherosclerosis.5 Data from knockout models suggest the importance of PPARγ and -δ for the anti-inflammatory M2 macrophage polarization and prevention of insulin resistance in the adipose tissue or the liver.6–8 Mechanisms underlying anti-atherogenic and anti-inflammatory effects of PPARs may vary among the isoforms. Thus, elevated cholesterol efflux through the activation of the liver X receptor (LXR)—ATP-binding cassette (ABC) A1 axis9,10 and inhibition of co-repressor release from promoters of toll-like receptor activated genes11 are considered...
to be of importance for the anti-atherogenic and anti-inflammatory PPARγ actions in macrophages.

The role of PPARγ in human macrophages is less clear. Initial studies showed that PPARγ facilitates monocyte to macrophage differentiation and uptake of oxidized lipoproteins, leading to foam cell formation. Later, PPARγ activation in human monocytes was shown to promote the generation of M2 polarized macrophages. However, the role of PPARγ in M2 polarization is controversial and it was postulated that PPARγ activation may be a target, but not a driver, for M2 polarization. Furthermore, some PPARγ target genes, such as CD36 and fatty acid-binding protein 4 (FABP4), may have deleterious roles during the development of atherosclerosis or insulin resistance. How the expression of PPARγ target genes affects macrophage polarization in the context of metabolic disease requires further research.

5-Aminoimidazole-4-carboxamide riboside (AICAR, acadesine) is a purine nucleoside analogue, which is tested clinically for the treatment of cardiovascular and haematological diseases, including ischaemia/reperfusion or chronic and acute lymphoblastic leukaemia. In cells AICAR is phosphorylated by adenosine kinase to 5-aminoimidazole-4-carboxamide ribotide (ZMP). ZMP activates AMP-dependent protein kinase (AMPK), a serine/threonine kinase regulated by the levels of intracellular AMP/ADP. AMPK is a major sensor of cellular energy depletion. Its activation restores energy homeostasis by activating catabolic processes such as glycolysis and fatty acid oxidation, while inhibiting anabolic processes such as protein and fatty acid synthesis. In macrophages, AMPK supports M2 polarization and prevents macrophage inflammatory responses to saturated fatty acids (SFA) such as palmitate, by activating protein deacetylase Sirt1 or reducing palmitate-induced inflammation activation through stimulating mitophagy. Although many biological actions of AICAR are mediated by AMPK, AICAR also exerts AMPK-independent effects. Thus, AICAR induces apoptosis of chronic lymphocytic leukaemia cells by up-regulating pro-apoptotic Bcl-2 family proteins and suppresses tissue factor expression through the activation of the phosphoinoside 3-kinase/Akt pathway. In macrophages, AICAR inhibits lipopolysaccharide-induced inductive nitric oxide synthase and cyclooxygenase-2 expression through direct interference with DNA binding of CREB, C/EBPβ, and NFκB transcription factors. The influence of AICAR/AMPK on monocyte to macrophage differentiation and changes of macrophage lipid metabolism are unknown.

In this work, we investigated the effects of AICAR during the differentiation of monocytic THP-1 cells and primary human monocytes to macrophages. In differentiating monocytes, AICAR suppressed PPARγ protein induction and downstream target gene expression in an AMPK-independent fashion. This was associated with attenuated macrophage inflammatory and endoplasmic reticulum (ER)-stress responses to oxidized lipoproteins and SFA, suggesting that an interference with PPARγ activation during monocyte differentiation contributes to beneficial cardiometabolic effects of AICAR.

2. Methods

2.1 Cell culture and fatty acid treatment

THP-1 human acute monocytic leukaemia cells were cultured in RPMI 1640 medium supplemented with 10% foetal calf serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Macrophage differentiation of THP-1 cells was induced by treatment with 100 nM phorbol 12-myristate 13-acetate (PMA). Human monocytes were isolated from commercially obtained buffy coats (provided by DRK-Blutspendedienst Baden-Württemberg-Hessen, Institut für Transfusionsmedizin und Immunhämatologie, Frankfurt, Germany) by using ficoll density centrifugation followed by magnetic separation with positive selection (CD14 MicroBeads, Miltenyi Biotec). Monocytes were differentiated into macrophages by culture in macrophage serum-free medium (Invitrogen) containing 50 ng/mL human recombinant macrophage colony-stimulating factor (M-CSF) (Peprotech). Palmitate was prepared by diluting 100 mM stock solution in 70% ethanol/0.1 M NaOH into 10% fatty acid-free low endotoxin bovine serum albumin (BSA) (A-8806, Sigma-Aldrich) to obtain a palmitate:BSA molar ratio of 3:1 followed by adjustment to pH 7.4. BSA was used in control incubations.

2.2 LDL isolation and treatment

Human LDL (d = 1.02–1.06 g/mL) was isolated from the plasma of healthy volunteers by sequential ultracentrifugation. Modification of LDL by oxidation was performed by incubating LDL with 5 μM CuSO4 at room temperature for 24 h followed by dialysis against PBS with 100 μM EDTA.

2.3 PPARγ and AMPKα1 knockdown

To create THP-1 cell lines with a stable PPARγ and AMPKα1, cells were infected with lentiviruses coding for PPARγ and AMPKα1 shRNA or non-targeting control shRNA (MISSION shRNA, Sigma). Lentivirally transduced THP-1 clones were selected by culture in medium containing 1 μg/mL puromycin.

2.4 Quantitative PCR

Total RNA from 1 × 10⁶ THP-1 cells or human primary monocytes was isolated using the PeqGold RNApure kit (PeqLab) and transcribed using the cDNA Synthesis kit (Fermentas). Quantitative PCR was performed with iQ SYBR green Supermix (Bio-Rad) using the CFX96 system from Bio-Rad. Primer sequences for quantitative PCR can be obtained upon request. Expression was normalized to actin.

2.5 Western blot

Protein lysates from 1 × 10⁶ THP-1 cells or human primary monocytes were resolved on 7.5–15% polyacrylamide gels followed by transfer onto nitrocellulose or polyvinylidene difluoride (for FABP4) membrane. Membranes were incubated with antibodies against FABP4 (sc-18661, Santa Cruz Biotechnology), PPARγ (#2443), phospho-eukaryotic translation initiation factor 2-α (eIF2α) (Ser51) (#3998), phospho-Jun (Ser73) (#3270), phospho-acyl-CoA carboxylase (ACC) (Ser79) (#3661), phospho-Raptor (Ser792) (#2083), phospho-S6 (Ser240/244) (#2215), ACC (#3662), AMPK (#2793), raptor (#2280) (all Cell Signaling Technology), CD36 (FA6–152, Fisher Scientific), actin (A-2066, Sigma-Aldrich) or nucleolin (sc-13057, Santa Cruz Biotechnology), followed by IRDye 680 and IRDye 800-coupled secondary antibodies (LI-COR Biosciences). Blots were visualized and quantified using the Odyssey imaging system (LI-COR Biosciences). Band intensities were normalized to actin or nucleolin.

2.6 Chromatin immunoprecipitation

Chromatin immunoprecipitation was performed according to Schmidl et al. THP-1 cells treated with PMA and AICAR were crosslinked for 10 min with 1% formaldehyde and extracts were sonicated until the DNA fragments were 300–800 bp in average size. Crosslinked chromatin was immunoprecipitated with 2.5 μg of PPARγ antibody (H-100X, Santa Cruz Biotechnology) or rabbit IgG overnight at 4 °C followed by incubation with protein A-agarose beads (Santa Cruz Biotechnology) for an additional 2 h. Immunoprecipitated DNA was recovered using the PCR purification kit (Qiagen) and analysed using Q-PCR. Primers corresponding
to macrophage PPAR-responsive element (PPRE) of the human FABP4 promoter were used for analysis. Data are shown as a percentage of input DNA precipitated by a corresponding antibody.

2.7 Dil-oxLDL uptake
Oxidized LDL (oxLDL) (250 µg/mL) was labelled with 1 mM 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI) by overnight incubation at 37°C. THP-1 cells or primary human macrophages were incubated with 20 µg/mL Dil-oxLDL for 3 and 6 h, followed by the analysis of Dil-oxLDL uptake using flow cytometry (LSRFortessa, BD Biosciences). Data are presented as a median fluorescence intensity of 10,000 cell events.

2.8 CCL18 ELISA
Analysis of CCL18 secretion from primary monocytes was performed using an ELISA kit from RayBiotech according to manufacturer instructions.

2.9 Statistical analysis
Data are presented as means ± SEM of at least three independent experiments. Data were analysed by the one-way ANOVA test with Bonferroni post hoc means comparison using the OriginPro 8.5G software (OriginLab). Differences were considered statistically significant for P < 0.05.

3. Results
Searching for genes involved in fatty acid metabolism and responding to AMPK activators in differentiating THP-1 cells revealed FABP4 (adipocyte fatty acid-binding protein, aP2) to be suppressed by AICAR. Inducing monocyte differentiation with PMA strongly elevated FABP4 mRNA (Figure 1A) and protein (Figure 1B) levels, which was concentration-dependently inhibited by co-treatment with AICAR. To prove that the inhibitory effect of AICAR on FABP4 induction is not restricted to cell lines, we used primary human monocytes, differentiated in the presence of M-CSF. Induction of FABP4 mRNA and protein during primary monocyte differentiation was also inhibited by AICAR in a concentration-dependent fashion (Figure 1C and D).

Although AICAR is commonly used as an AMPK activator, it is known to have AMPK-independent effects. To elucidate whether suppression of FABP4 by AICAR during monocyte differentiation is AMPK mediated, we analysed the impact of other AMPK activators. Metformin and A-769662 are distinct AMPK activators working either by altering the energy status of the cell or by a direct allosteric effect. In contrast to AICAR, metformin and A-769662 failed to suppress FABP4 protein in PMA-treated THP-1 cells (Figure 2A).

To verify AICAR effects to be independent of AMPK, we created THP-1 lines with a stable knockdown of AMPKα1. Western analysis confirmed strongly reduced AMPKα1 expression in these cells (Figure 2B), whereas the AMPK catalytic subunit AMPKα2 was undetectable in THP-1 cells at the mRNA and protein level (data not shown). Functionality of the knockdown was verified by analysing AMPK substrate phosphorylation. Basal and AICAR-stimulated phosphorylation of the AMPK substrates ACC and raptor were reduced in AMPKα1 knockdown cells (Figure 2B). However, AICAR still inhibited PMA-induced FABP4 mRNA expression in AMPKα1-silenced THP-1 cells.
cells (Figure 2C), providing further evidence that the AICAR effect is AMPK independent.

To activate AMPK, intracellular AICAR is phosphorylated by adenosine kinase to ZMP. To assess whether conversion of AICAR to ZMP is necessary to inhibit FABP4 induction, we incubated differentiating THP-1 cells with AICAR in the presence of the adenosine kinase inhibitor 5-iodotubercidin. This treatment fully blocked AMPK activation by AICAR, as followed by phosphorylation of ACC (Figure 2D). However, the AICAR effect on FABP4 persisted in the presence of 5-iodotubercidin (Figure 2E), suggesting that the conversion of AICAR to ZMP is not required for inhibition of FABP4 expression.

We then questioned whether cellular AICAR uptake is a prerequisite for its action on FABP4. Cells take up AICAR through adenosine transporters. Thus, we incubated differentiating THP-1 cells with AICAR in the presence of the adenosine transporter inhibitor dipyridamole. Dipyridamole abolished AMPK activation as followed by ACC phosphorylation (Figure 2D) and restored FABP4 expression (Figure 2E). Thus, intracellular accumulation of AICAR is needed to suppress PMA-induced FABP4 expression.

Induction of FABP4 via PPARγ is a common feature of macrophage and adipocyte differentiation. Triggering a differentiation programme in pre-adipocytes initially activates the transcription factors C/EBPα and C/EBPβ, thus increasing the expression of C/EBPα and PPARγ, which in turn activates the expression of genes involved in lipid metabolism, including FABP4. AICAR is known to interrupt adipocyte differentiation upstream of PPARγ and C/EBPα induction.27,28 As PPARγ is also induced during monocyte differentiation, we evaluated the effects of AICAR on PPARγ mRNA expression. Figure 3 shows that the expression of PPARγ mRNA during THP-1 or primary monocyte differentiation was not inhibited by AICAR (Figure 3).

The divergent effects of AICAR on FABP4 and PPARγ mRNA expression suggested that AICAR may act at the level of, or downstream of PPARγ, interfering with PPARγ-dependent gene expression. To test this, we analysed the mRNA expression of genes known to be activated by PPARγ during monocyte differentiation. The PPARγ target genes CD36 and lipoprotein lipase were induced during THP-1 or primary monocyte differentiation, and their induction was inhibited in the presence of AICAR (Figure 3). Interestingly, not all PPARγ target mRNAs were inhibited. The expression of cholesterol transporter ABCA1 was increased in AICAR-treated THP-1 cells as well as in primary monocytes.

Next, we asked how AICAR affects PPARγ-dependent gene expression. Therefore, we analysed the impact of AICAR on PPARγ protein expression. Figure 4A shows that AICAR time-dependently

Figure 2 AICAR signals independently of AMPK. (A) FABP4 protein expression in THP-1 cells incubated with PMA, 2 mM metformin, or 100 µM A-769662 for 24 h. *P < 0.05 vs. PMA. (B) Western analysis and quantification of phospho-ACC, -raptor, and AMPK protein in THP-1 cells transduced with control virus (CV) or AMPKα1 shRNA (KD) and treated with 1 mM AICAR for 24 h. NS, non-specific band. (C) FABP4 mRNA expression in CV and AMPKα1 KD cells treated with PMA and AICAR for 24 h. *P < 0.05 vs. CV. (D and E) ACC phosphorylation (D) and FABP4 mRNA expression (E) in THP-1 cells treated for 24 h with PMA, AICAR, 10 µM dipyridamole (Dip), or 0.5 µM 5-iodotubercidin (5-Iodo). *P < 0.05 vs. AICAR, #P < 0.05 vs. untreated cells.

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Similarly, AICAR prevented PPARγ inhibition by blocking protein synthesis. To confirm that reduced PPARγ protein in AICAR-treated cells results in diminished PPARγ recruitment to its target gene promoters, we performed chromatin immunoprecipitation (ChIP) experiments using a PPAR-response element at the FABP4 promoter as a probe. Figure 4D shows that in untreated cells no specific association of PPARγ with FABP4 PPRE could be detected, while differentiation with PMA increased PPARγ occupancy of FABP4 PPRE, which was significantly inhibited by the co-treatment with AICAR.

AICAR-activated AMPK may interfere with cap-dependent translation by targeting the TORC1 pathway. To test whether inhibition of TORC1 influences PPARγ protein levels, we treated THP-1 cells with PMA in the presence of the TORC1 inhibitor rapamycin or the AMPK activator phenformin. Inhibition of TORC1 was confirmed by analysing phosphorylation of the TORC1 downstream target S6 ribosomal protein (Figure 4E). In contrast to AICAR, neither rapamycin nor phenformin affected PPARγ protein levels, indicating that AICAR effects on PPARγ protein are independent of interfering with cap-dependent translation.

Several PPARγ target genes regulate lipid storage and metabolism. In the context of atherosclerosis, PPARγ targets such as CD36 and FABP4 appear to be pro-atherogenic, facilitating the uptake of modified lipoproteins and increasing inflammation. It was shown that PPARγ activation during monocyte differentiation facilitates oxLDL uptake. Thus, we asked whether inhibition of PPARγ target gene expression in differentiating macrophages by AICAR affects macrophage responses to oxLDL. To avoid potential interferences with direct anti-inflammatory effects of AICAR-activated AMPK, we incubated differentiated THP-1 cells for 24 h in the absence of PMA and AICAR (recovery) prior to oxLDL treatment. Under these conditions AMPK activity, followed by phosphorylation of ACC, had returned to a basal level, whereas the expression of FABP4 and CD36 proteins remained inhibited (Figure 5A). Next, we studied the effect of AICAR on the macrophage uptake of oxLDL labelled with the fluorescent lipophylic dye Dil (Dil-oxLDL) using flow cytometry. As shown in Figure 5B, the presence of AICAR during differentiation time-dependently reduced the uptake of Dil-oxLDL in macrophages. We then examined the effect of AICAR on oxLDL-induced activation of c-Jun N-terminal kinase (JNK), which plays a central role in macrophage responses to oxLDL and is known to be activated by oxLDL in a CD36-dependent fashion. Figure 5C shows that oxLDL increased JNK activity in THP-1 cells as analysed by phosphorylation of the JNK substrate c-Jun. This effect was attenuated when THP-1 cells were differentiated in the presence of AICAR.

FABP4 has been shown to facilitate macrophage pro-inflammatory and ER-stress responses to SFA. Thus, we questioned whether the presence of AICAR during monocyte differentiation influences JNK activation and ER-stress markers such as phosphorylated eIF2α upon palmitate treatment. As presented in figure 5D, AICAR suppressed JNK activation and an ER-stress response to SFA in differentiated THP-1 cells. To confirm that PPARγ activation during monocyte differentiation supports pro-inflammatory responses to SFA in macrophages, we analysed palmitate effects in THP-1 cells with a stable knockdown of PPARγ (PPARγ KD), which exhibited ~70–80% reduction in PPARγ and its target FABP4 proteins upon differentiation (data not shown). Figure 5E shows that palmitate-induced phosphorylation of eIF2α and c-Jun was slightly, but significantly, inhibited in PPARγ KD cells. Conversely, when differentiation was carried out in the presence of inhibited PPARγ protein accumulation in differentiating THP-1 cells. Similarly, AICAR prevented PPARγ protein induction in differentiating primary monocytes (Figure 4A). To study the effect of AICAR on PPARγ protein stability, we pre-treated cells for 16 h with PMA in the absence or presence of AICAR, followed by blocking protein synthesis with cycloheximide. Figure 4B shows that PPARγ protein is rapidly degraded with a half-life time of ~1 h. However, the rate of PPARγ degradation was not affected by AICAR. When analysing PPARγ protein synthesis by blocking protein degradation with the proteasome inhibitor MG132, we observed that AICAR prevented PPARγ protein accumulation in MG132-treated cells (Figure 4C).
the synthetic PPARγ ligand rosiglitazone, we observed significantly increased eIF2α and c-Jun phosphorylation (Figure 5F). To corroborate these findings in primary cells, we differentiated primary human monocytes for 6 days in the presence of 0.5 mM AICAR or 10 μM the PPARγ antagonist GW9662, followed by 24 h incubations without AICAR and GW9662. These cells were then analysed for the uptake of Dil-oxLDL or the ER stress response to oxLDL and palmitate. Figure 5G shows that macrophages differentiated in the presence of AICAR failed to take up Dil-oxLDL. In addition both, AICAR and GW9662, when present during monocyte differentiation, significantly reduced mRNA of CHOP, an ER stress-marker, upon oxLDL or palmitate treatment (Figure 5H).

PPARγ activation was reported to be required for M2 polarization of mouse and human macrophages. However, recent studies suggested that, although PPARγ is induced during IL-4-triggered macrophage M2 polarization, it is not directly required for the expression of IL-4-dependent genes. To investigate whether AICAR affects IL-4-induced gene expression in differentiating monocytes, we treated human primary monocytes with M-CSF and IL-4 in the absence or presence of AICAR. As shown in Figure 6A, AICAR did not inhibit the IL-4-induced mRNA expression of genes encoding the mannose receptor (Mrcl/CD206), chemokine (C-C motif) ligand 18 (CCL18/AMAC1), or monoamine oxidase (MAO-A). Similarly, IL-4-induced secretion of CCL18 protein was not affected by AICAR (Figure 6B). These data suggest that the induction of macrophage M2 polarization is not compromised by AICAR.

4. Discussion

In this work, we show that AICAR suppresses PPARγ protein expression during monocyte differentiation. This alters PPARγ target gene activation to reduce oxLDL uptake and to attenuate macrophage
inflammatory responses to oxLDL and SFA. We propose a mechanism whereby AICAR affects macrophage polarization under conditions relevant for metabolic disease in addition to previously reported AMPK-dependent effects, such as inhibition of NFκB, activation of Sirt1 or increased β-oxidation.19,20,33 Remarkably, our data support the notion that AICAR signals independently of AMPK or its conversion to ZMP, which is necessary to activate AMPK or to provoke transcriptional activation by AICAR in yeast.34 Our data contrast previous findings suggesting the involvement of AMPK in PPARγ inhibition in rat hepatoma cells.25 It should be noted that PPARγ protein levels were not affected by AMPK in that study, implicating alternative modes of PPARα/γ regulation by AMPK in a cell- or species-specific manner. Previous work showed that AICAR inhibits CREB-, C/EBPβ-, and NFκB-mediated transcription in macrophages by interfering with binding of these transcription factors to the responsive elements of their target genes.24 Although this mechanism may affect PPARγ-dependent gene expression besides regulating PPARγ protein, we could not observe an altered DNA binding property of PPARγ when AICAR was added to nuclear extracts in vitro in EMSA experiments (data not shown). Rather, our data suggest that AICAR reduces PPARγ target gene promoter occupancy primarily by reducing PPARγ protein amount.

The mechanism how AICAR reduces PPARγ protein expression requires further clarification. Experiments using cycloheximide, the
proteasomal inhibitor MG132, and rapamycin suggest that AICAR is unlikely to affect PPARγ protein stability and probably interferes with PPARγ translation in a way, which does not involve cap-dependent translation. This is different from previously published data suggesting regulation of PPARγ protein in adipocytes by Map4k4 kinase through a rapamycin-sensitive pathway. One possible mechanism may be the induction of microRNAs, such as miR-27b, which was reported to inhibit PPARγ protein levels without affecting its transcript in some, but not all studies. However, AICAR did not affect miR-27b expression in our experimental system (data not shown). Alternatively, AICAR may be envisioned to affect cap-independent translation, such as IRES-dependent translation, which is insensitive to rapamycin treatment.

PPARγ is considered to have anti-atherogenic and anti-inflammatory properties in macrophages. Data supporting this role are derived from studies using ligand-induced PPARγ activation in differentiated macrophages as well as knockout mouse models. Less data exist regarding the role of PPARγ in differentiating human monocytes. Initial work suggested PPARγ to support foam cell formation in human monocytes with the notion that PPARγ induces the scavenger receptor CD36, a mechanism confirmed also in PPARγ knockout macrophages. This pro-atherogenic effect was later contrasted by the finding that PPARγ stimulates cholesterol efflux through LXR-dependent induction of the cholesterol transporters ABCA1 and ABCG1. Remarkably, our data show that AICAR inhibits CD36 expression, while stimulating ABCA1 expression, thereby creating an anti-atherogenic macrophage phenotype. Reduced foam cell formation in AICAR pre-treated macrophages was confirmed in Dil-oxLDL uptake experiments. ABCA1 up-regulation by AICAR occurred independently of LXR activation as the mRNA expression of LXRα/β and its target ABCG1 were down-regulated in the presence of AICAR (data not shown). Thus, it is likely that AICAR activates ABCA1 expression in a PPARγ/LXR-independent fashion, by a mechanism, which merits further investigation. It is also notable that FABP4, the PPARγ target gene showing the highest induction, has a spectrum of pro-inflammatory and pro-atherogenic actions in macrophages. Our data show that AICAR suppresses FABP4 expression in differentiating THP-1 cells and primary monocytes, suggesting that reduced FABP4 may contribute to the attenuated pro-inflammatory responses of macrophages to oxLDL or palmitate. Additional long lasting AMPK-dependent effects, related to activation of sirtuins, increased oxidative metabolism, or autophagy, may also support an anti-inflammatory phenotype of macrophages differentiated in the presence of AICAR. In contrast, a direct interference of AICAR-activated AMPK with inflammatory signalling induced by palmitate or oxLDL is less likely, since AICAR was removed from the system upon differentiation. Furthermore, our data using PPARγ knockdown cells indicate that PPARγ in differentiating monocytes contributes to pro-inflammatory responses of macrophages to palmitate, suggesting a link between AICAR-induced PPARγ down-regulation and reduced inflammatory and ER-stress responses of macrophages to SFA.

PPARγ supports M2 macrophage polarization as suggested in a number of studies in the murine as well as the human system. However, the link between PPARγ and M2 polarization may be less pronounced as recently suggested. Our data show that AICAR does not interfere with IL-4-induced polarization of monocytes and even increases some IL-4-dependent target genes. Interestingly, IL-4-polarized macrophages were reported to exhibit reduced cholesterol accumulation in spite of decreased ABCA1 expression. Future studies should determine whether AMPK activation further alters the phenotype of IL-4-polarized macrophages towards reduced foam cell formation.

In conclusion, we suggest that AICAR inhibits PPARγ-dependent gene expression during monocyte to macrophage differentiation and alters macrophage lipid metabolism to reduce foam cell formation, thus, attenuating pro-inflammatory responses to modified lipoproteins or SFA. These effects may contribute to the spectrum of beneficial cardiometabolic actions attributed to AICAR.

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