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Rosiglitazone Inhibits Monocyte/Macrophage Adhesion Through De Novo Adiponectin Production in Human Monocytes

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ABSTRACT

Rosiglitazone (RSG) has a variety of actions on both insulin sensitization and anti-atherogenic effects. The molecular effect of RSG on monocyte/macrophage function in terms of de novo synthesis of adiponectin is not fully understood. Here, we examined the regulation of adiponectin expression in human monocytes/macrophages by RSG and its function on monocyte adhesion during initiation of atherosclerosis. Adiponectin expression in monocytes and macrophages was studied by RT-PCR, quantitative real-time PCR, Western blot, and immunocytochemistry. Signal transduction and adhesion molecules were studied in order to describe the function of de novo synthesized adiponectin in monocyte adhesion. Adiponectin was expressed and upregulated during monocyte differentiation. The expression of adiponectin was enhanced, albeit at a much lesser degree, by a peroxisome proliferator-activated receptor gamma (PPARγ) agonist RSG, which was similar to what was found in adipocytes. Monocyte adhesion was remarkably reduced when the cells were treated with RSG for 12 h. This inhibitory effect of RSG was abolished by specific anti-adiponectin antibodies but not by non-immune immunoglobulin G in a serum-free condition. Adiponectin-induced suppression on monocyte adhesion was inhibited by a selective AMP-activated protein kinase (AMPK) inhibitor compound C. The reduced expression and/or function of adhesion molecule integrins may underlie the mechanism contributing to reduced monocyte adhesion upon AMPK activation. Our data suggest that the inhibitory effect of RSG on monocyte adhesion might be at least in part through de novo adiponectin expression and activation of an AMPK-dependent pathway, which might play an important role in atherogenesis. J. Cell. Biochem. 110: 1410–1419, 2010.

KEY WORDS: ADIPONECTIN; DE NOVO EXPRESSION; AMP-ACTIVATED PROTEIN KINASE; MONOCYTE ADHESION; PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA (PPARγ) AGONIST

Rosiglitazone (RSG), one of thiazolidinediones which target at peroxisome proliferator-activated receptor gamma (PPARγ), has been recently used as an anti-hyperglycemic agent. However, several lines of studies have shown that RSG can modulate a variety of cardiovascular risk factors, in addition to its glucose-lowering and lipid-modifying effects [Choi et al., 2004; Marx et al., 2005]. Therefore, RSG not only increases insulin sensitivity but also has anti-inflammatory, anti-atherogenic, and anti-thrombotic properties [Lehrke and Lazar, 2005].

Abbreviations used: AICAR, 5-aminoimidazole-4-carboxamide riboside; AMPK, AMP-activated protein kinase; CS-1, connecting segment-1; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde dehydrogenase; HAECs, human aortic endothelial cells; ICH, immunocytochemistry; IL-4, interleukin-4; MMP, matrix metalloproteinase; PBMCs, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PMA, phorbol 12-myristate 13-acetate; PPARγ, peroxisome proliferator-activated receptor gamma; RSG, rosiglitazone; RT-PCR, reverse transcriptase-polymerase chain reaction; SDS–PAGE, sodium dodecylsulfate–polyacrylamide gel electrophoresis; TNFα, tumor necrosis factor-alpha; VCAM-1, vascular cell adhesion molecule-1.

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There are several proposed mechanisms to explain underlying pathways leading to anti-atherogenic and anti-thrombotic activities of PPARγ agonists [Touyz and Schiffrin, 2006]. For example, in endothelial cells, PPARγ agonists inhibit expression of interleukin-1β, interleukin-6, and tumor necrosis factor-alpha (TNFα), and attenuate TNFα-induced intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) expression [Bruemmer et al., 2005]. PPARγ activators can also act as vasorelaxants through enhanced endothelial NO production [Calnek et al., 2003]. PPARγ activators enhance the expression of PPARγ in macrophages and inhibit synthesis of scavenger receptor A and matrix metalloproteinase (MMP)-9 [Ricote et al., 1999]. PPARγ activators also target at vascular smooth muscle cells, where they exert inhibitory effects on proliferation and migration, oxidative stress, AT1R expression, and release of matrix-degrading enzymes [Law and Hsueh, 2001; Diep et al., 2002; Bruemmer et al., 2005], and modulate DNA replication/cell-cycle progression of the vascular smooth muscle cells [Bruemmer et al., 2005].

One more likely explanation of the effect of PPARγ agonist is through its adiponectin-raising effect as shown in many clinical studies [Maeda et al., 2001; Yang et al., 2002a]. We and others have shown a negative correlation between circulating levels of adiponectin and obesity, insulin resistance, coronary artery disease, and dyslipidemia [Havel, 2002; Yang et al., 2002b]. Increased level of adiponectin by bariatric surgery in patients with morbid obesity clearly demonstrated an improvement of insulin sensitivity as measured by modified insulin suppression test [Yang et al., 2001]. In adiponectin-deficient mice, neointimal thickening is exacerbated in the damaged arteries; this pathological change can be ameliorated by replacement of adiponectin [Kubota et al., 2002]. Therefore, through PPARγ activation, the RSG-induced adiponectin expression in adipocytes may underlie its pharmacological functions, as adiponectin contributing to insulin-sensitizing and anti-atherogenic effects is well established [Lihn et al., 2005].

Previous studies demonstrated that the effects of adiponectin on monocyte/macrophage cells are primarily through inhibition of the expression of endothelial cell adhesion molecules. For example, adiponectin dose-dependently inhibited expression of VCAM-1, E-selectin, and ICAM-1 in THP-1 as well as TNFα-induced THP-1 adhesion on human aortic endothelial cells (HAECs) [Ouchi et al., 1999]. Although adiponectin has been considered to be expressed and secreted largely from the adipose tissue, adiponectin mRNA expression has been detected in several other cell types, including primary hepatic sinusoidal endothelial cells, stellate cells, and macrophages [Wolf et al., 2006]. In this study, we examine the effect of RSG on the expression, regulation, and function of de novo synthesized adiponectin in monocytes/macrophages and the mechanism of the monocyte/macrophage-produced adiponectin on monocyte adhesion, a very early process of atherosclerosis.

MATERIALS AND METHODS

CELL PREPARATION AND CULTURE

Human peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation on a Ficoll-sodium metrizoate density gradient (Amersham Biosciences, Uppsala, Sweden), according to the manufacturer's instructions. After washing with phosphate-buffered saline (PBS), the cells were suspended in RPMI 1640 medium (Gibco) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated FBS (Biological Industries). Human monocytes were isolated from PBMC by collecting the attached cells [Mantuano et al., 2007], which were differentiated into macrophages by treatment with IL-4 (Peprotech EC LTD) (10 ng/ml) for 3 days [Huang et al., 1999]. This study was approved by the Ethical Review Board of the National Taiwan University Hospital. Written informed consent was obtained from all participating subjects.

The THP-1 human monocytic cell line (ATCC, TIB-202) was maintained in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal bovine serum. Differentiation of THP-1 cells into macrophages was obtained by treatment with PMA (Sigma, St Louis, MS) (10^{-7} M) for 3 days [Ramprasad et al., 1996].

SEMIQUANTITATIVE RT-PCR ANALYSIS

Total RNA was extracted by REzol (PROtech Technology, Sparks, NV), according to the manufacturer's instructions. Single-stranded cDNA was synthesized with SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA). The primer sets for amplification of adiponectin genes (sense, 5′-GCG GCC GCG CCT ATG TAT ACC GCT CAG CA-3′; anti-sense, 5′-GGA TCC TCA GTT GGT GTC ATG GTA GAG A-3′) and PPARγ (sense, 5′-TTC TAC TTT GAT CGC ACT TTG GT-3′; anti-sense, 5′-TCT GGG AGA TTC TCC TGT TGA C-3′) were synthesized. Semiquantitative RT-PCR was performed with an initial denaturation at 94°C for 5 min, followed by denaturing at 94°C for 30 s, annealing at 62°C for 30 s, and polymerization at 72°C for 30 s for a total of 35 cycles, then by a final extension at 72°C for 10 min. The expression levels of mRNA were normalized by the median expression of the housekeeping gene glyceraldehyde dehydrogenase (GAPDH). The PCR products were resolved on agarose gel electrophoresis.

QUANTITATIVE REAL-TIME PCR ANALYSIS

RNA was isolated with REzol and then performed with high capacity RNA-to-cDNA Kit (Applied Biosystem, Carlsbad, CA) for cDNA synthesis. cDNAs were amplified in a 20 μl reaction volume containing TaqMan gene expression master mix (Applied Biosystem) according to the manufacturer's instructions. The Q-PCR was performed with ABI 7000 Real-Time PCR system, with primers for measuring adiponectin (forward: 5′-AGA AAG GAG ATC CAG GTC TTA TTG GT-3′, reverse: 5′-AAC GTA AGT CTC CAA TCC CAC ACT-3′) and integrin (Hs00164957_m1, NM_000211.2 from ABI Biosystems). The cDNA concentration in each sample was normalized using transcripts for GAPDH (Hs99999905_m1 and NM_002046.3 from ABI Biosystems).

WESTERN BLOT ANALYSIS

Cells were harvested at the indicated time points of each experiment, after which whole cell lysates were dissolved in NP40-containing lysis buffer [Sharma et al., 2007]. To detect adiponectin, 80 μg of

whole cell lysates was resolved with sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Proteins were then transferred onto Hybond-P membrane (Amersham Biosciences, Arlington Heights, IL). Western blotting was performed according to the procedure supplied with the ECL Western blotting reagents kit (Amersham Biosciences, IL). The anti-adiponectin antibody was purchased from R&D Systems (Minneapolis), anti-PPARγ antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and both anti-AMPK and anti-pAMPK antibodies were purchased from Cell Signaling Technology (Danvers, MA). Antibody for β-actin was used as an internal control.

MONOCYTE ADHESION ASSAY

Monocytes were cultured in serum-free medium with or without RSG (13.5 μM) for 12 h. The 96-well plate was incubated overnight with fibronectin (Roche, Penzberg, Germany) at a concentration of 10 μg/ml [Richter et al., 2005]. Monocytes were suspended at the concentration of 4×10^5 cells per well and incubated for 2 h. To assess the effects of adiponectin on monocyte adhesiveness, the monocytes were pre-incubated for 30 min with exogenous adiponectin (BioVision, Mountain View, CA) with or without an AMP-dependent protein kinase (AMPK) inhibitor compound C (Merck) before they were transferred to the fibronectin-coated plates. An AMPK activator, 5-aminoimidazole-4-carboxamide riboside (AICAR; Cell Signaling Technology), and the AMPK inhibitor compound C from Merck [Zhou et al., 2001] were used as positive controls in the assays. The adherent cells were then stained with crystal violet [Chana et al., 2003] dissolved in ethanol and sodium citrate (0.1 M) (1:1 volume ratio) after three washes of PBS to remove the non-adherent cells. The optical density, measured by a spectrophotometer at a wavelength of 550 nm, was used as a measurement of the number of cells per well that were adhered to the fibronectin-coated surface.

IMMUNOCYTOCHEMISTRY

For immunocytochemistry (ICH) studies, cells adhered to fibronectin-coated cover glasses were treated with 4% paraformaldehyde for 15 min. After treatment with 0.1% Triton X-100 for 1 min, they were treated with bovine serum albumin in PBS (5 mg/ml) for 1 h to block non-specific binding. The cells were incubated with adiponectin (1:50 dilution; R&D Systems), HAM56 (1:50 dilution; Dako, Carpinteria, CA), or integrin (α_5) (1:200 dilution; Santa Cruz Biotechnology) antibodies for 1 h at 37°C. They were then incubated with FITC-conjugated or rhodamine-conjugated secondary antibodies (1:100 dilutions; Sigma) for 1 h at room temperature, and stained with DAPI (1:3,000 dilutions) for 15 min. The cells were then observed by confocal fluorescent microscopy (EZ-C1; Nikon, Tokyo, Japan). Negative control was performed by omitting the incubation of the cells with primary antibodies.

STATISTICAL ANALYSIS

Data were expressed as means ± SE. Differences between groups were analyzed by one-way ANOVA and Bonferroni's posttest. Trends across ordered groups were analyzed by the non-parametric trend test. A two-tailed $P < 0.05$ was the threshold for significance. Statistical analysis was performed with STATA 9.0 statistical software (StataCorp., TX).

RESULTS

EXPRESSION AND REGULATION OF PPARγ AND ADIPONECTIN IN HUMAN MONOCYTES AND MACROPHAGES

To study the effect of RSG, expressions of the PPARγ and adiponectin were studied in monocyte/macrophage cells. As shown in Figure 1A, both PPARγ and adiponectin mRNAs were found in monocytes isolated from human PBMCs. The expression levels were highly increased when monocytes were induced to macrophages by treatment of IL-4 (10 ng/ml) for 3 days. To verify the species of mRNA for adiponectin, the cloned cDNA fragments obtained from the circulating monocytes/macrophages or the cultured THP-1 cells

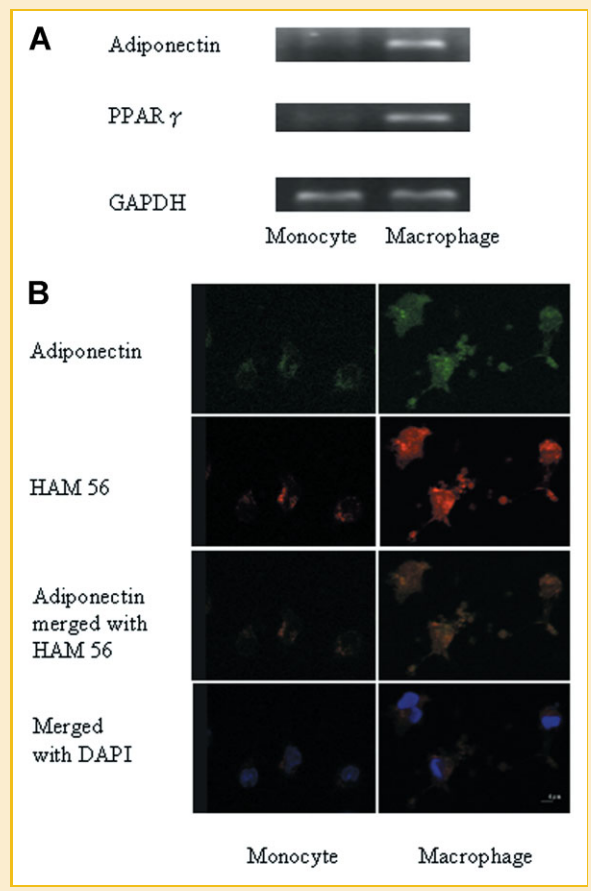

Fig. 1. Expression of adiponectin mRNA and protein in human monocytes/macrophages. The expression of adiponectin mRNA and PPARγ mRNA was examined by RT-PCR in human monocytes and macrophages under the treatment of IL-4 (10 ng/ml) for 3 days. GAPDH was used as the internal control (A). The expression of adiponectin (in green) presented in the cytoplasm of human monocytes and macrophages by immunocytochemistry. HAM56 (red) and DAPI (blue) staining were used as monocyte/macrophage and nuclear markers, respectively (B). The merged images of adiponectin staining and those of HAM56 and DAPI were shown on the bottom. The expression of adiponectin was mainly localized in the cytoplasm, and its level was higher in macrophages than that in monocytes. Scale bar = 4 μm.

were directly sequenced and the results revealed a 100% homology with the mRNA sequence of adiponectin, thus confirming the expression of adiponectin (data not shown). To illustrate the expression and cellular localization of the de novo synthesized adiponectin protein in monocytes/macrophages, an ICH study was undertaken. As shown in Figure 1B, we used HAM 56 staining for monocyte/macrophage cell lineage which indicated higher cytoplasmic expression of HAM 56 in macrophages. The staining pattern of adiponectin was similar to that of HAM 56, showing adiponectin expression was mainly present in the cytoplasm of monocytes and, with a greater intensity, in macrophages.

EFFECT OF RSG ON EXPRESSION AND FUNCTION OF PPARγ AND ADIPONECTIN IN HUMAN MONOCYTES AND MACROPHAGES

To further study the molecular mechanisms and function of RSG on monocyte functions, we studied the expression of PPARγ and adiponectin in the THP-1 cells. The protein expression of adiponectin and PPARγ increased during differentiation of macrophages, reaching a maximum at the third day following PMA (10^{-7} M) treatment (Fig. 2A). We compared the relative expression levels of PPARγ and adiponectin, and found that though PPARγ levels were comparable, the expression levels of adiponectin in monocytes and macrophages were far less than that expressed in adipocytes (Fig. 2B).

Following treatment of RSG for 24 h in the monocytes, the expressions of PPARγ and adiponectin were upregulated in a dose-dependent manner (upper panel, Fig. 2C), reaching a near twofold increase in the adiponectin protein levels (lower panel, Fig. 2C). Similarly, adiponectin was also upregulated by RSG in macrophages (data not shown). The RSG-mediated increase of adiponectin expression was blocked dose-dependently by a PPARγ antagonist treatment for 24 h, GW 9662 in monocytes (Fig. 2D). Further experiments by Western blot analysis to detect secreted adiponectin in the serum-free supernatant also showed that adiponectin levels

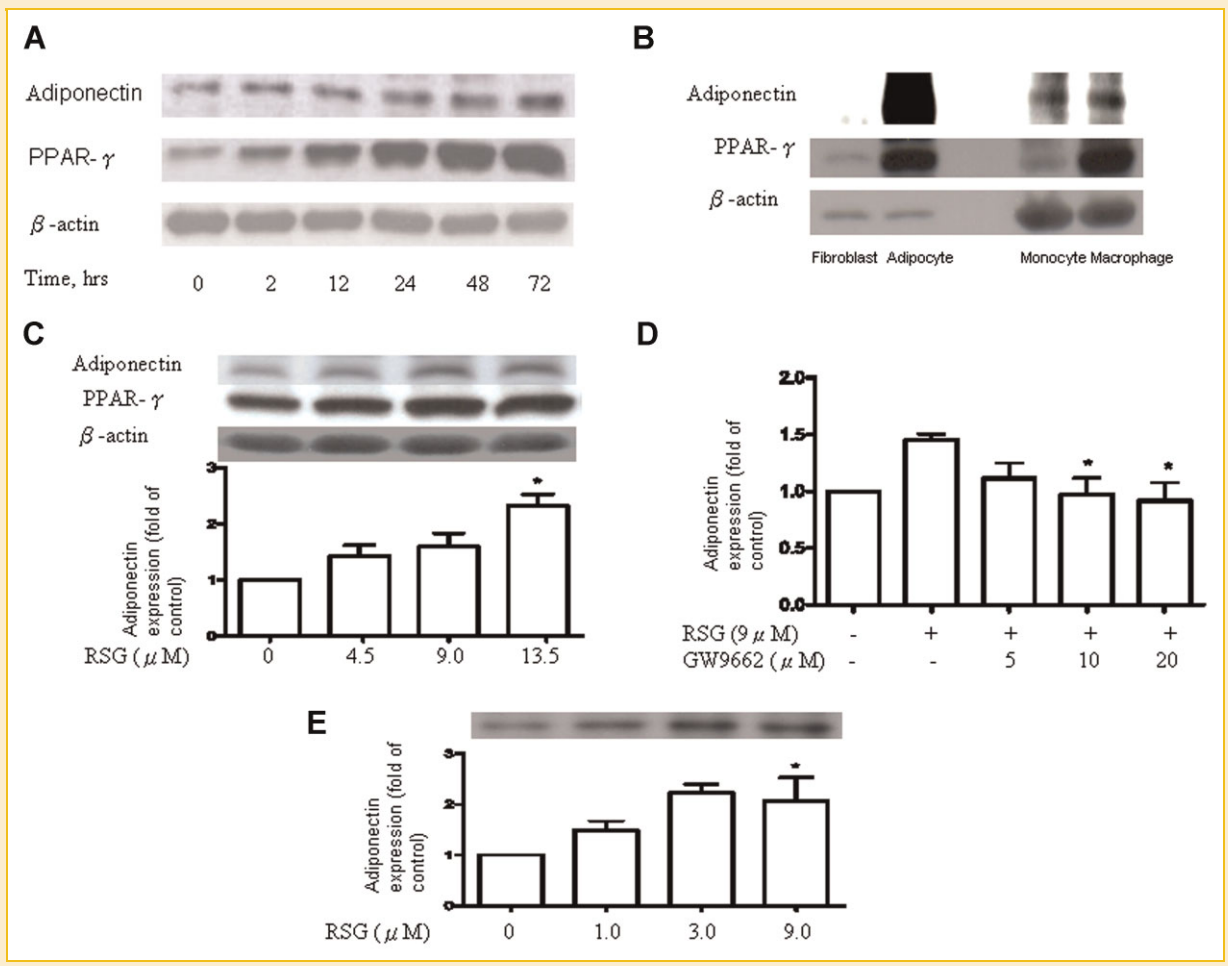

Fig. 2. Regulation of adiponectin expression in THP-1 monocytes and macrophages. The expression of adiponectin is upregulated in THP-1 cells stimulated by PMA (10^{-7} M) (A). The expression levels of adiponectin in monocytes/macrophages are much lower when compared with those of adipocytes (B). The expression of adiponectin in monocytes is enhanced by RSG (PPAR agonist) in a dose-dependent manner (C). Based on statistical analysis, there was a significant increase in adiponectin expression in monocytes with treatment of RSG (n = 3, *P* for trend <0.01) (C). PPARγ antagonist GW 9662 abolished the RSG-stimulated adiponectin production in a dose-dependent manner (n = 4, *P* for trend = 0.01) (D). To detect the adiponectin levels in the serum-free supernatant, we concentrated the volume of supernatant from 600 to 30 μl by Vivaspin 2 concentrators (Sartorius Stedim Biotech, Goettingen, Germany) following treatment of RSG for 12 h. Adiponectin levels increased in a dose-dependent manner (n = 3, *P* for trend = 0.01) (E). $^{*}P < 0.05$; $^{**}P < 0.01$.

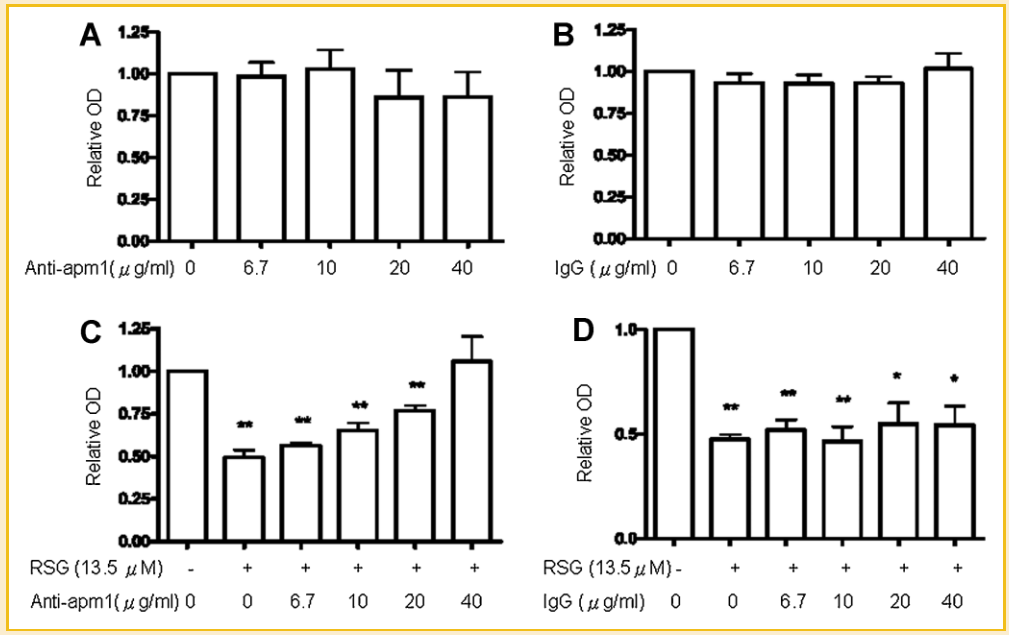

Fig. 3. De novo synthesized adiponectin contributes to the inhibition on monocyte adhesion to fibronectin. Monocyte adhesion to fibronectin-coated (10 μg/ml) wells was assessed in the cells treated without (A,B) or with (C,D) RSG (13.5 μM) for 12 h. Control cells incubated either with specific anti-adiponectin antibodies or with non-specific IgG did not affect cell adhesion to fibronectin-coated wells (A,B). Cells treated with RSG, adhesion to fibronectin-coated wells is significantly reduced (C,D). The RSG-induced inhibition on monocyte adhesion is reverted by the addition of the specific anti-adiponectin antibodies (C, n = 5, *P* for trend <0.05) but not by non-specific IgG (D, n = 4). $^{*}P < 0.05$; $^{**}P < 0.01$.

increased in a dose-dependent manner following treatment of RSG for 12 h (Fig. 2E).

To study the biological function of the adiponectin expressed in monocytes, we employed an assay of monocyte adhesion to fibronectin-coated wells in a serum-free condition. In the absence of RSG, the monocyte adhesion was not affected by incubation with either anti-adiponectin antibody (Fig. 3A) or non-immune IgG (Fig. 3B). However, when the monocytes were treated with RSG to enhance adiponectin expression, their adhesion to fibronectin-coated wells was significantly reduced (Fig. 3C). To confirm the role of adiponectin in RSG-mediated monocyte adhesion inhibition, we analyzed monocyte adhesion in the presence of either specific anti-adiponectin antibody or non-specific IgG. As shown in Figure 3C, the inhibitory effect of monocyte adhesion was abolished by the specific anti-adiponectin antibodies in a concentration-dependent manner, but not affected by the non-immune IgG (Fig. 3D). Together, these data indicate that the RSG-mediated inhibition on monocyte adhesion to fibronectin-coated wells is, at least in part, mediated by the de novo synthesized adiponectin in these monocytes.

SIGNALING PATHWAYS UNDERLYING ADIPONECTIN-MEDIATED SUPPRESSION ON MONOCYTE ADHESION

Since the previous study had shown the ability of adiponectin to activate AMP-activated protein kinase (AMPK) in myocytes and hepatocytes [Yamauchi et al., 2002], we explored the effect of AMPK on monocyte adhesion. In the monocyte/macrophage lineage THP-1 cells, we found that both adiponectin and AICAR, an AMPK activator, enhanced the phosphorylation of the Thr^{172} residue of AMPK in a time- and dose-dependent manner (Fig. 4A–D). The effects were blocked by a synthetic AMPK inhibitor, compound C (data not shown).

We then examined the role of AMPK phosphorylation on monocyte adhesion to fibronectin-coated wells. The results showed that the monocyte adhesion was reduced dose-dependently by adiponectin or AICAR treatment (Fig. 5A,B), suggesting that activation of AMPK signaling pathway might be involved in the inhibition of monocyte adhesion. Indeed, these inhibitory effects were abolished in the presence of an AMPK inhibitor, compound C (Fig. 5C,D). These data demonstrate that the adiponectin-induced suppression on monocyte adhesion was mediated via activation of AMPK signaling pathway.

As extracellular matrix proteins and adhesion molecules, including fibronectin and integrin ($\alpha_5\beta_1$), play important roles in cell adhesion [Dougherty et al., 1988; Schmidt and Kao, 2007], we studied the effect of AMPK activation on regulation of integrin. The expression of integrin (α_5) was presented in the cytoplasm, with stronger staining located near the plasma membranes of adhered cells on fibronectin-coated cover glasses as shown by the ICH study (Fig. 6A). Upon treatment with either adiponectin (10 μg/ml) or AICAR (500 μM) for 2 h, the fluorescence staining of integrin decreased when compared with that of non-treated control cells (Fig. 6A). We also confirmed the results with Western blot analysis. Both adiponectin (Fig. 6B) and AICAR treatment (Fig. 6C) inhibited the fibronectin-induced integrin expression in a dose-dependent manner. To ascertain the role of AMPK, we found that the AMPK inhibitor, compound C, abolished the inhibitory action of AICAR on fibronectin expression in a dose-dependent manner (Fig. 6D).

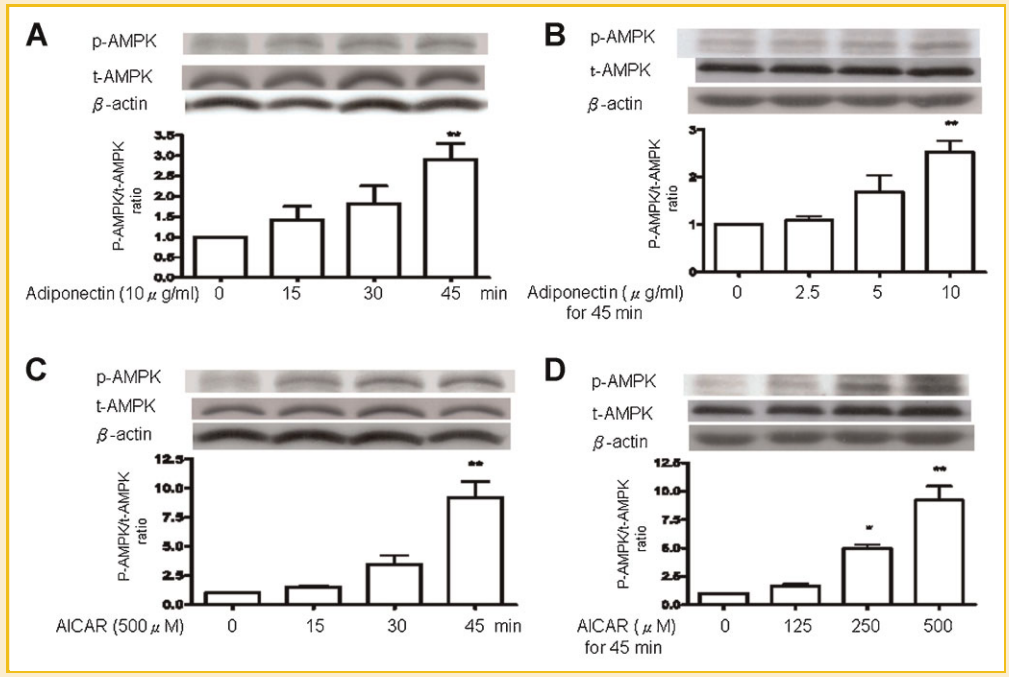

Fig. 4. Both AICAR and adiponectin increase AMPK phosphorylation. Adiponectin increases phosphorylation of AMPK in THP-1 monocytes in a time- and dose-dependent manner (A, n = 5, *P* for trend <0.01) (B, n = 3, *P* for trend <0.05), as well as the AMPK activator AICAR (n = 3, *P* for trend <0.05) (C,D). $^{*}P < 0.05$; $^{**}P < 0.01$.

DISCUSSION

In this study, we demonstrated for the first time that RSG upregulates PPARγ and adiponectin in human monocytes. We further demonstrated that de novo synthesized adiponectin in monocytes/macrophages plays a role in preventing monocyte adhesion to fibronectin-coated wells via the AMPK-dependent inhibition on integrin expression.

The adiponectin expression in human monocytes increased during monocytes differentiation to macrophages. The expression of

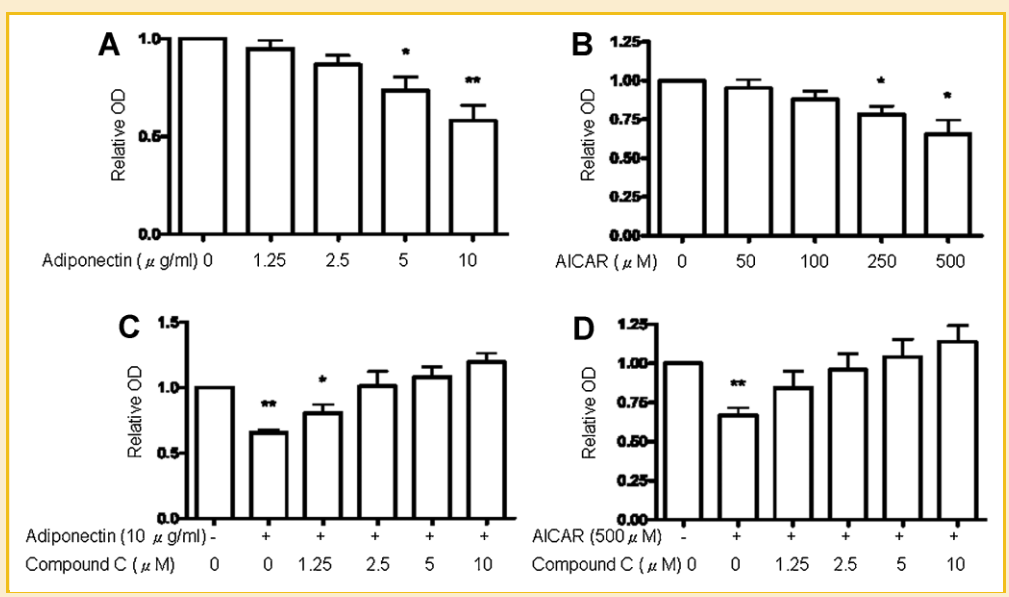

Fig. 5. AMPK regulates adiponectin-inhibited monocyte adhesion to fibronectin. Adiponectin (A, n = 5, *P* for trend <0.05), as well as an AMPK activator AICAR (B, n = 5, *P* for trend <0.05), dose-dependently reduces monocyte adhesion to fibronectin-coated wells. This inhibitory effect is blocked by an AMPK inhibitor compound C in a dose-dependent manner for both adiponectin (C, n = 5, *P* for trend <0.05) and AICAR (D, n = 5, *P* for trend <0.05). $^{*}P < 0.05$; $^{**}P < 0.01$.

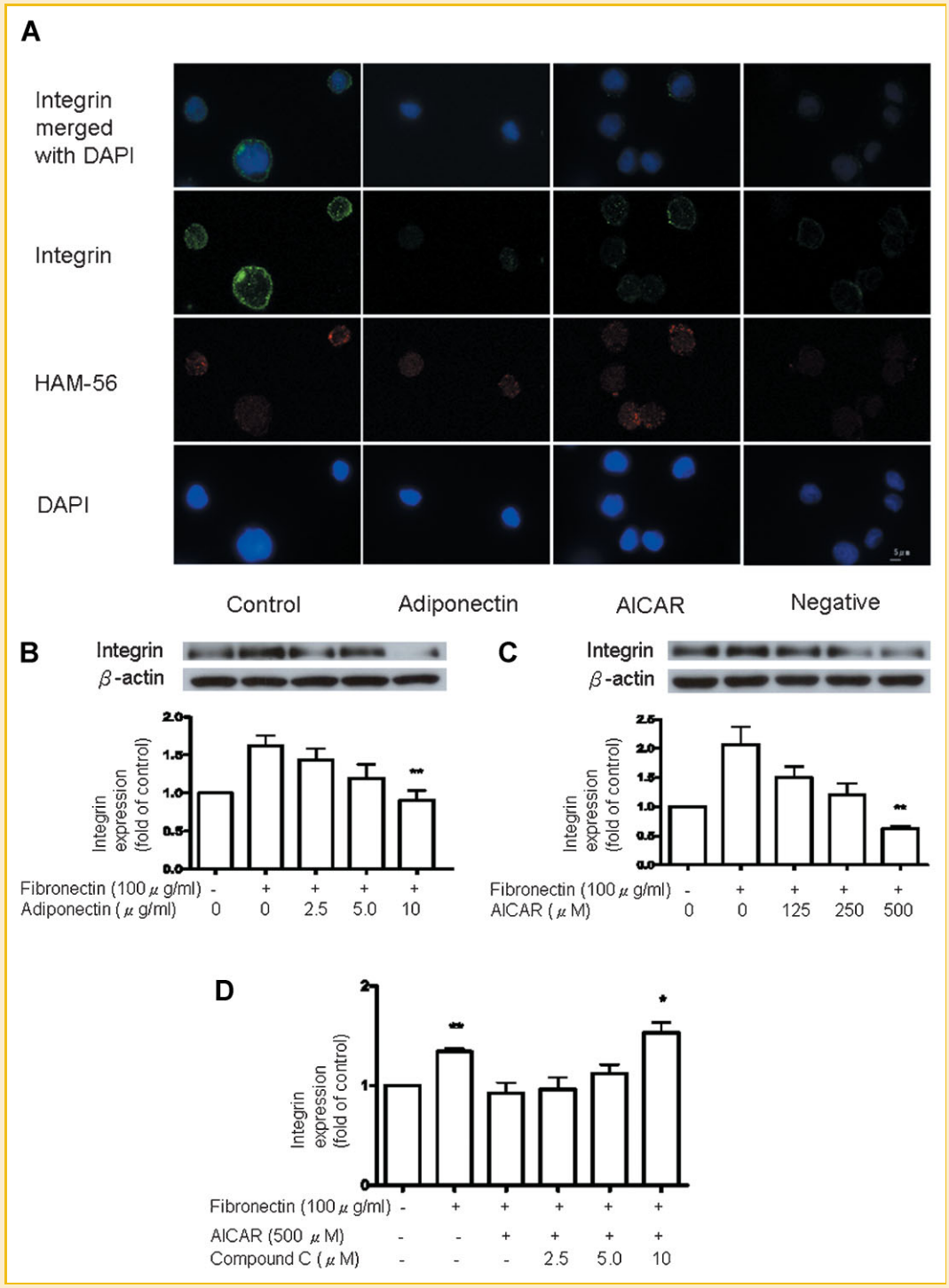

Fig. 6. Adiponectin and AICAR treatment decreases the fibronectin-induced expression of integrin in monocytes. The expression of integrin is mainly localized in the cytoplasm of untreated control cells. Upon treatment with adiponectin (10 μg/ml) or AICAR (500 μM) for 2 h, the expression of integrins is reduced, whereas there is no difference in the expression of HAM-56 (red). Nuclei were marked by DAPI staining (blue). Negative panel was performed by omitting the incubation of the cells with primary antibodies (A). Scale bars = 5 μm. To confirm the results with Western blot analysis, monocytes were pre-treated with adiponectin or AICAR in various doses for 30 min, and then incubated with fibronectin (100 μg/ml) for 9 h. Both adiponectin (B, n = 5, *P* for trend <0.05) and AICAR (C, n = 3, *P* for trend <0.05) inhibited the fibronectin-induced integrin expression in a dose-dependent manner. An AMPK inhibitor compound C abolishes the inhibitory action of AICAR in a dose-dependent manner (n = 3, *P* for trend = 0.01) (D). $^{*}P < 0.05$; $^{**}P < 0.01$.

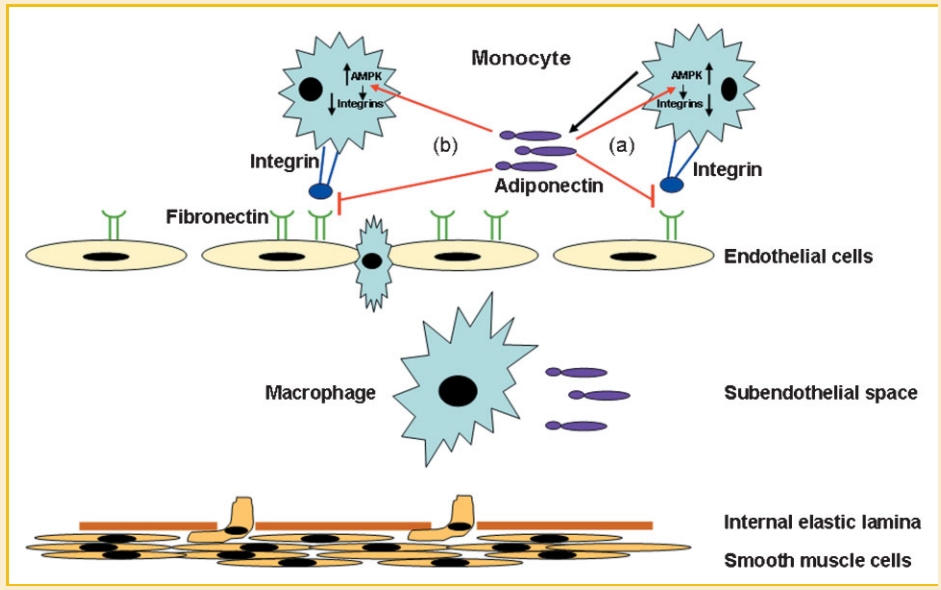

Fig. 7. Model of action of de novo synthesized adiponectin on monocytes/macrophages. De novo synthesized adiponectin inhibits monocyte adhesion to fibronectin via AMPK-dependent inhibition on integrin expression in an autocrine (a) or paracrine (b) fashion.

adiponectin was closely correlated with that of PPARγ during monocyte differentiation into macrophages. In addition, the induced adiponectin expression by RSG is blocked by a PPARγ antagonist GW 9662, suggesting that the expression of adiponectin is under PPARγ regulation at the level of gene expression. Recent studies have demonstrated that adiponectin expression was present in non-adipose tissues under certain pathological conditions, such as in liver and myotubes [Delaigle et al., 2004; Wolf et al., 2006]. The function of the locally produced adiponectin was viewed as a local anti-inflammatory protection. Although adiponectin is expressed in monocytes/macrophages, its level is relatively low. However, the locally produced adiponectin by monocytes exerted a remarkable effect of reduction on cellular adhesion to fibronectin-coated wells, suggesting that the monocyte-produced adiponectin may function via an autocrine or paracrine fashion. These findings not only expand our understanding of the initial steps of atherosclerosis but also provide a potential avenue for therapeutic intervention on atherosclerotic diseases in humans.

Monocyte adhesion to extracellular matrix proteins on endothelial surface has been considered as the major early step in the initiation of atherosclerosis [Huo et al., 2000; Vogl-Willis and Edwards, 2004]. For example, both soluble plasma and insoluble cellular fibronectin, as a known ligand to integrin receptor ($\alpha_5\beta_1$) of monocytes [Schmidt and Kao, 2007], can activate the integrin signaling pathway that leads to cell adhesion, cell migration, and morphogenesis [Miyamoto et al., 1998]. In vivo, fibronectin, a major fraction of which is plasma-derived, is deposited in the atherosclerosis-prone sites before other signs of atherosclerosis [Orr et al., 2005; Moretti et al., 2007]. In addition, binding of integrin receptors on monocytes with connecting segment-1 (CS-1) fibronectin expressed on endothelium plays an important role in mediating the entry of monocytes into human atherosclerotic lesions [Cole et al., 2003; Srinivasan et al., 2003]. Earlier studies employing adenovirus-mediated delivery or addition of recombinant adiponectin proteins have demonstrated that adiponectin possesses inhibitory effect on monocyte adhesion to endothelial cells via suppression of expression of adhesion molecules of the endothelial cells, including VCAM-1, endothelial-leukocyte adhesion molecule-1 (E-selectin), and ICAM-1 [Ouchi et al., 1999]. On the other hand, we in this study demonstrated the remarkable effect of RSG on monocyte/macrophages by showing increased expression of PPARγ and adiponectin, which in turn down-regulated the expression of integrin via an AMPK-dependent pathway. Thus, our finding may explain how RSG disrupt the interaction between fibronectin and integrin, and thus the cell adhesion activity. Based on these data, we propose a working model for the role of de novo synthesized adiponectin in monocytes/macrophages. This model elucidates that locally produced adiponectin may exert its inhibitory effect on integrin expression and thus reduce monocyte/macrophage adhesion in an autocrine or paracrine fashion (Fig. 7).

In summary, we provided new evidence to show that RSG can upregulate the expression and function of PPARγ and adiponectin in human monocytes/macrophages. Furthermore, locally regulated expression of adiponectin by the PPARγ agonist RSG may inhibit monocyte adhesion to fibronectin-coated wells via activation of AMPK signaling pathway and reduce the expression of the adhesion molecule integrins.

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