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Gliclazide inhibits differentiation-associated biologic events in human monocyte-derived macrophages

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Abstract

We investigated the in vitro effect of gliclazide on human monocyte-derived macrophage scavenger receptor expression and activity, foam cell formation, and lipopolysaccharide-induced cytokine production. Differentiation of human monocytes into macrophages in the presence of gliclazide (1-10 μg/mL) decreased CD36 expression by 20% to 50%, with maximal effect occurring at 2.5 μg/mL ($P < .05$). This effect was mimicked by vitamin E (50 μmol/L) and *N*-acetyl-L-cysteine (10 mmol/L). Incubation of the cells with gliclazide and *N*-acetyl-L-cysteine also reduced CD36 activity by 30% ($P < .02$). Despite these effects, neither gliclazide nor vitamin E did affect foam cell formation. In contrast, gliclazide significantly reduced lipopolysaccharide-stimulated macrophage tumor necrosis factor α and interleukin 6 secretion ($P < .05$). Overall, these data indicate that gliclazide, at concentrations in the therapeutic range, may regulate some key biologic events associated with the process of monocyte differentiation into macrophages.

1. Introduction

Patients with type 2 diabetes mellitus are at high risk of developing cardiovascular diseases, including coronary artery, peripheral arterial, and cerebrovascular diseases [1]. Epidemiologic studies have shown that these patients have a 2- to 4-fold increase in the risk of coronary and peripheral arterial diseases [2]. Oxidative stress has been suggested as one major contributor to long-term diabetic vascular complications [3]. Possible mechanisms involved in oxidative stress-induced vascular complications include diacylglycerol and protein kinase C activation [4].

Despite the apparent clinical failure of antioxidants in secondary prevention of coronary heart disease [5], pharmacologic management of oxidative stress may demonstrate benefit in diabetic patients. Indeed, antioxidant agents have been shown to inhibit low-density lipoprotein (LDL) oxidation, isoprostane generation, and monocyte superoxide anion release in these patients [6,7]. A great deal of evidence indicates that gliclazide, a sulfonylurea widely used in the treatment of type 2 diabetic patients, exhibits antioxidant properties [8-11]. We and others have demonstrated that this drug modulates vascular cell function through its antioxidant properties [12-16]. Of particular interest, we have demonstrated that gliclazide inhibits agonist-induced monocyte adhesion to endothelial cells [17,18]. Because monocyte differentiation into macrophages and foam cell formation are key events in the progression of atherosclerosis [19], the present study was conducted to investigate whether gliclazide may modulate these processes. Furthermore, based on previous evidence demonstrating that the process of monocyte differentiation into macrophages is associated with enhanced sensitivity toward lipopolysaccharide (LPS) [20], we also investigated the effect of gliclazide on LPS-stimulated tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) secretion by monocyte-derived macrophages.

2. Materials and methods

2.1. Reagents

Oil red O, LPS, vitamin E, glyburide, and monoclonal anti–β-actin (mouse immunoglobulin G1 isotype) were

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purchased from Sigma (St Louis, MO). *N*-acetyl-L-cysteine (NAC) was obtained from Calbiochem (La Jolla, CA). TRizol reagent, phosphate-buffered saline (PBS), Hanks balanced salt solution (HBSS), and RPMI 1640 medium were obtained from GIBCO (Grand Island, NY). Penicillin-streptomycin, fetal bovine serum, trypsin, and EDTA were obtained from Wisent (St Bruno, Quebec, Canada). Les Laboratoires Servier (Neuilly, France) supplied sodium salt gliclazide. Polyclonal antibody to CD36 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). 1,1′-Dioctadecyl-3,3,3′,3′ -tetramethylindocarbocyanine perchlorate (DiI) was purchased from Molecular Probes (Eugene, OR).

2.2. Isolation of human monocytes

Human monocytes were isolated from fresh heparinized blood (100 mL) collected from nonsmoker healthy male and female donors as previously described [21]. First, peripheral blood mononuclear cells were obtained by density centrifugation using Ficoll (Amersham Pharmacia, Baie D'Urfé, Quebec, Canada). The cells collected from the interface were washed 3 times with HBSS and allowed to aggregate in the presence of fetal bovine serum. After further purification by rosetting technique and density centrifugation, recovery of highly purified monocytes (85%-90%), as assessed by fluorescence-activated cell sorter analysis, was obtained using phycoerythrin-conjugated anti-CD14 monoclonal antibody (Becton Dickinson, Oakville, ON, Canada). Human monocytes were resuspended in serum-free RPMI 1640 medium supplemented with 1% (vol/vol) penicillin-streptomycin. Monocytes were allowed to differentiate into macrophages by culture for 9 days at 37°C in the presence of 10% autologous serum. Incubation with gliclazide was conducted for 9 days, that is, the period required for the complete differentiation of monocytes into macrophages.

2.3. Isolation of human LDL

LDL was isolated from plasma obtained from healthy nonsmoker normolipidemic male and female donors. Venous blood collected into tubes containing EDTA and LDL (density = 1.019-1.063) was isolated by sequential ultracentrifugation using potassium bromide for density adjustment. Low-density lipoprotein was sterilized by filtration through 0.20 μmol/L Gelman filters (Gelman Sciences, Ann Arbor, MI). Minimally modified LDL (mmLDL) was obtained by storage of EDTA-free LDL at 4°C for at least 3 months. Protein content of LDL preparations was determined using the Bradford method with BSA as standard [22]. Endotoxin content of LDL preparations (100 μg/mL) was determined by the Limulus amebocyte lysate assay (Sigma) and was consistently found to be lower than 3 pg/mL.

2.4. RNA isolation and complementary DNA preparation

Freshly isolated human monocytes were incubated under appropriate conditions for 9 days at 37°C. At the end of this incubation time, the cells were lysed with Trizol reagent, and total cytoplasmic RNA was extracted by the acid-phenol technique of Chomczynski and Sacchi [23], precipitated, and resuspended in diethyl pyrocarbonate water. Complementary DNA was synthesized from RNA by incubating 2 μg of total cellular RNA with 0.1 μg oligodT (Amersham Biosciences, Piscataway, NJ) for 5 minutes at 98°C. The mixture was then incubated for 60 minutes at 37°C and for 10 minutes at 99°C in reverse transcription mixture (Roche Diagnostics, Laval, QC, Canada).

2.5. Determination of CD36 messenger RNA expression

CD36 messenger RNA (mRNA) levels in monocyte-derived macrophages were assessed by PCR. Complementary DNA was amplified by using 2 synthetic primers specific for human CD36 (5′ -TGTAACCCAGGACGCTGAGGAC-3′ and 5′-CTGTACCATTAATCATGTCGCAGTGAC-3′). A 443–base pair human CD36 fragment was amplified enzymatically by 28 repeated cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 68°C for 1 minute in a programmable thermal controller (PTC-100, MJ Research Inc, Watertown, MA). A 456–base pair human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) complementary DNA was amplified by 20 amplification cycles at 98°C for 40 seconds, 60°C for 40 seconds, and 72°C for 90 seconds using 2 synthetic primers specific (5′-CCCTTCATTGACCTCAACTACATGG-3′ and 5′-AGTCTTCTGGGTGGCAGTGATGG-3′) for human GAPDH. The reaction products were visualized by electrophoresis on a 1% agarose gel containing 1μg/mL ethidium bromide. A 100–base pair DNA ladder (Fermentas, Flamborough, ON, Canada) was run to generate size markers. The integrated absorbance of the bands was measured with an image analysis scanning system (Alpha Imager 2000, Packard Instrument Company, Meriden, CT).

2.6. Measurement of TNF-α and IL-6 secretion by monocyte-derived macrophages

Freshly isolated human monocytes were differentiated in the presence or absence of gliclazide for 9 days. At the end of this incubation time, the cells were treated with 100 ng/mL LPS for 24 hours. Levels of TNF-α and IL-6 proteins in the culture medium were measured by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). The minimum detectable concentrations of TNF-α and IL-6 with these assays are typically less than 1.6 and 0.70 pg/mL, respectively.

2.7. Western blot analysis

Freshly isolated human monocytes were cultured under appropriate conditions for 9 days at 37°C. The cells were then lysed in radioimmuno-precipitation assay buffer containing 0.1% sodium dodecyl sulfate (SDS), 1% Nonidet P-40, and 0.5% Na-deoxycholic acid. Fifteen micrograms of total cellular proteins were separated by electrophoresis through a 10% SDS–polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane using a Trans-Blot

Cell system (Bio-Rad, Mississauga, ON, Canada). The membrane was blocked for 1 hour at room temperature with PBS containing 3% BSA. After 3 washes with PBS/Tween 20 (0.1%), the membrane was incubated overnight at 4°C with a rabbit polyclonal antibody to CD36 (1:1000) in PBS/Tween. The membrane was next washed with PBS/Tween and incubated for 1 hour at room temperature with a horseradish peroxidase–conjugated goat antirabbit immunoglobulin G (1:5000). Antigen detection was performed with an enhanced chemiluminescence detection system (Amersham Biosciences). The membranes were stripped and reprobed with 1:1000 dilution of a monoclonal antibody to human β-actin to correct for differences in protein loading.

2.8. Measurement of DiI-mmLDL uptake by human monocyte-derived macrophages

Freshly isolated human monocytes were cultured in 8-well culture slides (Falcon, Becton Dickinson) in the presence or absence of appropriate agents for 9 days at 37°C. The cells were then incubated with DiI-labeled mmLDL (80 μg/mL) for 3 hours in medium containing 5% of lipoprotein-deficient serum in the presence or absence of a 500-fold excess of unlabeled oxidized LDL (oxLDL). At the end of the incubation period, cells were washed, mounted on coverslips, and examined by fluorescence microscopy. The cells were seeded in 12-well plates and treated or not with

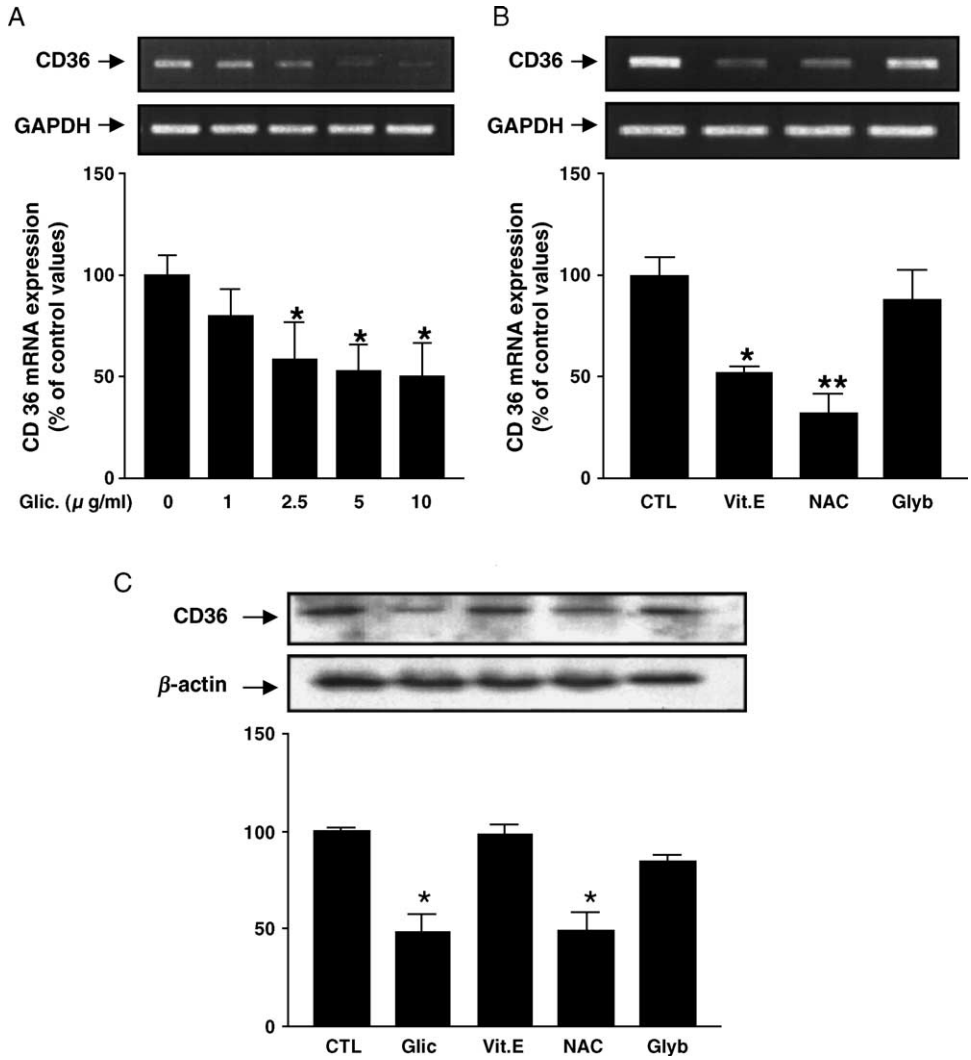

Fig. 1. Effect of gliclazide on CD36 mRNA expression by human monocyte-derived macrophages. Freshly isolated human monocytes were cultured in the presence or absence of increasing concentrations of gliclazide (1-10 μg/mL) (A), vitamin E (50 μmol/L), NAC (10 mmol/L), or glyburide (0.62 μg/mL) (B) for 9 days at 37°C. At the end of this incubation period, total RNA was extracted as described in Materials and Methods, and the levels of CD36 and GADPH mRNA expression were assessed by semiquantitative PCR. CD36 mRNA levels (arbitrary units), normalized to the levels of GAPDH mRNA expression, are presented. Data represent the mean ± SEM of 5 independent experiments. *$P < .05$ vs control. **$P < .01$ vs control. (C) Freshly isolated human monocytes were cultured in the presence or absence of gliclazide (10 μg/mL), vitamin E (50 μmol/L), NAC (10 mmol/L), or glyburide (0.62 μg/mL) for 9 days at 37°C. At the end of this incubation period, 15 μg of total cellular proteins were separated by SDS–polyacrylamide gel electrophoresis and subjected to Western blot as described in Materials and Methods. CD36 protein levels were normalized to the levels of β-actin protein. Data represent the mean ± SEM of 3 independent experiments. *$P < .05$ vs control.

appropriate agents to measure intracellular levels of DiI-mmLDL. At the end of the incubation period, DiI was extracted by isopropanol and the fluorescence was determined at excitation and emission wavelengths of 520 and 564 nm, respectively. Results were normalized to total cell protein concentrations.

2.9. Quantification of cellular lipid accumulation

Freshly isolated human monocytes were differentiated into macrophages under appropriate conditions for 9 days at 37°C. The cells were then treated with mmLDL (100 μg/mL) for 24 hours, and cellular lipid accumulation was quantified as previously described [24]. Briefly, cells were washed with PBS and fixed with 10% formalin solution for 1 hour at room temperature. They were next washed twice with PBS and stained with 0.5% oil red O in isopropanol for 3 hours at room temperature. Quantification of lipid accumulation was achieved by extracting oil red O from stained cells with isopropanol and measuring the optical density of the extract at 510 nm.

2.10. Determination of cell viability

To evaluate a possible cellular toxicity of gliclazide at the maximal concentration used in the study, cell viability was determined by trypan blue exclusion. It was consistently found to be higher than 95%.

2.11. Statistical analysis

Statistical analysis of the results was performed by 1-way analysis of variance followed by the Student-Newman-Keuls test for multiple comparisons or by the unpaired Student t test for pairwise comparisons. Differences were considered to be of statistical significance at $P < .05$. Results are expressed as the mean ± SEM.

3. Results

3.1. Effect of gliclazide on cell morphology during the process of monocyte differentiation into macrophages

Freshly isolated human monocytes were cultured for 9 days in RPMI 1640 supplemented with 10% autologous

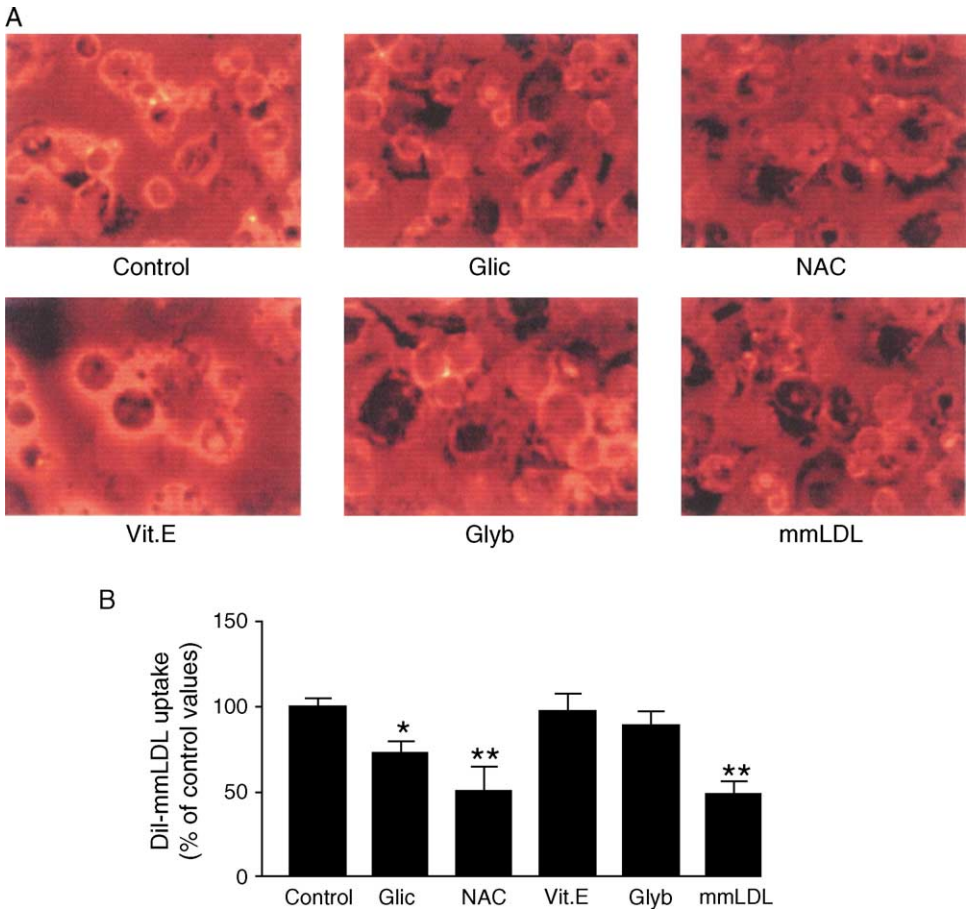

Fig. 2. Effect of gliclazide on DiI-mmLDL uptake by human monocyte-derived macrophages. Freshly isolated human monocytes were cultured in the presence or absence of gliclazide (10 μg/mL), vitamin E (50 μmol/L), NAC (10 mmol/L), or glyburide (0.62 μg/mL) for 9 days at 37°C. At the end of this incubation period, cells were exposed to DiI-mmLDL (80 μg/mL) for 3 hours in the presence or absence of excess unlabeled mmLDL. After washing, fluorescence of DiI was detected in the cytoplasm of monocyte-derived macrophages by fluorescence microscopy (A) or measured at excitation and emission wavelengths of 520 and 564 nm, respectively (B). Data illustrated on the graph bar represent the mean ± SEM of 5 independent experiments. *$P < .05$ vs control; **$P < .01$ vs control.

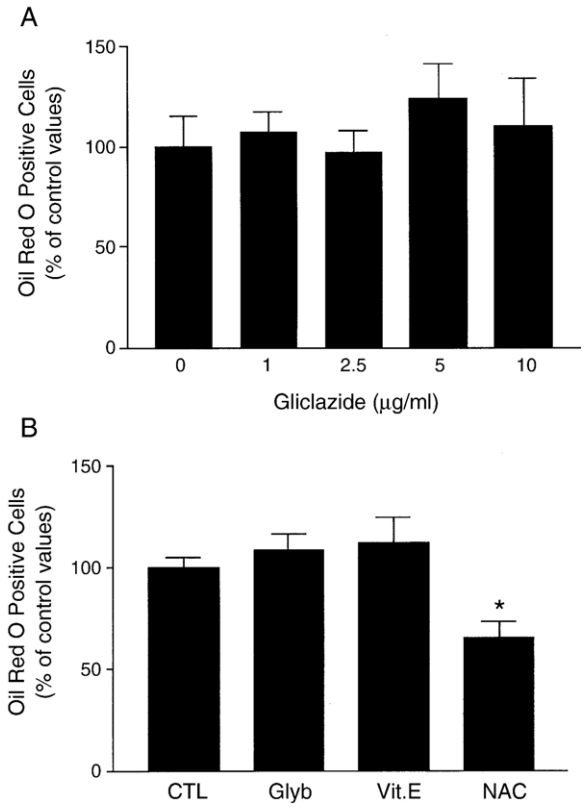

Fig. 3. Effect of gliclazide on foam cell formation induced by mmLDL. Freshly isolated human monocytes were cultured in the presence or absence of increasing concentrations of gliclazide (1-10 μg/mL) (A), vitamin E (50 μmol/L), NAC (10 mmol/L), or glyburide (0.62 μg/mL) (B) for 9 days at 37°C. At the end of this incubation period, foam cell formation was assessed by oil red O staining as described in Materials and Methods. Data represent the mean ± SEM of 6 independent experiments. *P < .05 vs control.

serum in the presence or absence of increasing concentrations of gliclazide (1-10 μg/mL) to investigate the effect of gliclazide on morphological differentiation of monocytes into macrophages. Human monocytes incubated in the presence of autologous serum readily differentiated into macrophages, as evidenced by development of cytoplasmic processes and cell flattening. Morphological differentiation of monocytes into macrophages was unaffected by gliclazide (data not shown).

3.2. Effect of gliclazide on CD36 expression by human monocyte-derived macrophages

Differentiation of freshly isolated human monocytes into macrophages in the presence of gliclazide resulted in a dose-dependent decrease in CD36 mRNA expression. Maximal decrease was observed at 10 μg/mL gliclazide (Fig. 1A). The inhibitory effect of gliclazide on CD36 mRNA levels was mimicked by vitamin E (50 μmol/L) and NAC (10 mmol/L). In contrast, equimolar concentrations of glyburide, a sulfonylurea without antioxidant properties (5 mg of glyburide equivalent to 80 mg of gliclazide) failed to inhibit macrophage CD36 expression (Fig. 1B). Reduction in CD36 mRNA levels was associated with a significant decrease in CD36 protein levels in gliclazide- and NAC-treated cells, but not in vitamin E- and glyburide-treated cells (Fig. 1C) (CD36 protein expression [% over basal values]: medium, 100 ± 2; gliclazide, 49 ± 9 [P < .05]; vitamin E, 99 ± 5; NAC, 50 ± 9 [P < .05]; glyburide, 85 ± 3).

3.3. Effect of gliclazide on DiI-mmLDL uptake by human monocyte-derived macrophages

Freshly isolated human monocytes were cultured in the presence or absence of this drug for 9 days to investigate the effect of gliclazide on CD36 activity, then exposed to DiI-mmLDL for 5 hours at 37°C. As shown in Fig. 2, gliclazide significantly decreased DiI-mmLDL uptake by human monocyte-derived macrophages (P < .05). Treatment of the cells with NAC (10 mmol/L) also resulted in a significant reduction in DiI-mmLDL uptake (P < .01). In contrast, vitamin E (50 μmol/L) and glyburide (0.62 μg/mL) had no significant effect on DiI-mmLDL uptake by the cells (mmLDL uptake [% over basal values]: medium, 99 ± 4.8; glyburide, 92 ± 11; gliclazide, 72 ± 7 [P < .05]; vitamin E, 95 ± 10; NAC, 50 ± 10 [P < .05]) (Fig. 2).

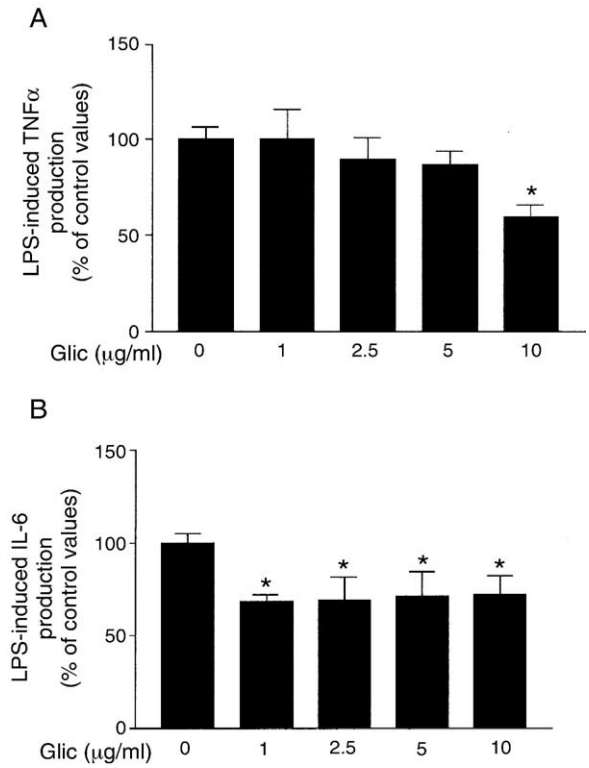

Fig. 4. Effect of gliclazide on LPS-stimulated TNF-α and IL-6 production by human monocyte-derived macrophages. Freshly isolated human monocytes were cultured in the presence or absence of increasing concentrations of gliclazide (1-10 μg/mL) for 9 days at 37°C. The cells were then exposed to LPS (100 ng/mL) for 24 hours. At the end of this incubation period, TNF-α (A) and IL-6 (B) levels in cell culture supernatants were determined by enzyme-linked immunosorbent assay. Data represent the mean ± SEM of 4 different experiments. *P < .05 vs control.

3.4. Effect of gliclazide on foam cell formation induced by mmLDL

Freshly isolated human monocytes were incubated in the presence or absence of gliclazide for 9 days to evaluate the effect of gliclazide on foam cell formation. The cells were then treated with mmLDL (100 μg/mL) for 24 hours at 37°C. Exposure of the cells to mmLDL resulted in intracellular lipid accumulation as assessed by oil red O staining. Addition of gliclazide in the culture medium during the process of monocyte differentiation into macrophages had no effect on this parameter (Fig. 3A). Likewise, culture of the cells in the presence of glyburide or vitamin E did not affect foam cell formation. In contrast, NAC (10 mmol/L) significantly inhibited mmLDL-induced foam cell formation (Fig. 3B).

3.5. Effect of gliclazide on LPS-stimulated TNF-α and IL-6 production by human monocyte-derived macrophages

Because the process of human monocyte differentiation into macrophages is associated with increased sensitivity to LPS [20], we next assessed the effect of gliclazide on LPS-induced TNF-α and IL-6 secretion by monocyte-derived macrophages. As shown in Fig. 4, presence of gliclazide in the culture medium significantly decreased LPS-induced production of these cytokines. Maximal effect of gliclazide on LPS-induced IL-6 and TNF-α secretion by monocyte-derived macrophages was observed at 1 and 10 μg/mL gliclazide, respectively. Glyburide and NAC mimicked the suppressive effect of gliclazide on LPS-induced TNF-α and IL-6 secretion by human macrophages, whereas vitamin E had no effect on these parameters (Fig. 5).

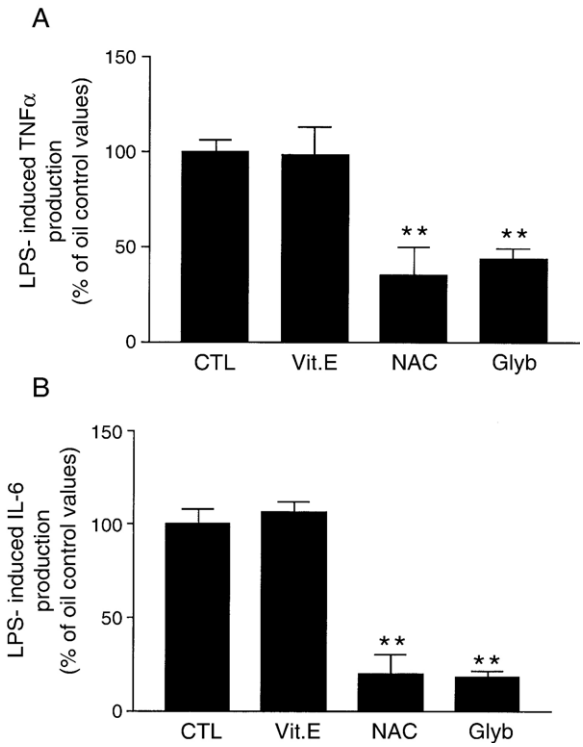

Fig. 5. Effect of vitamin E, NAC, and glyburide on LPS-stimulated TNF-α and IL-6 production by human monocyte-derived macrophages. Freshly isolated human monocytes were cultured in the presence or absence of vitamin E (50 μmol/L), NAC (10 mmol/L), or glyburide (0.62 μg/mL) for 9 days at 37°C. The cells were then treated with LPS (100 ng/mL) for 24 hours. At the end of this incubation period, TNF-α (A) and IL-6 (B) levels in cell culture supernatants were determined by enzyme-linked immunosorbent assay. Data represent the mean ± SEM of 4 different experiments. $^{**}P < .01$ vs control.

4. Discussion

Monocyte recruitment into the vascular wall and their differentiation into macrophages in the intima are key biologic events in atherogenesis [25]. As monocytes differentiate into macrophages, they show increased expression of the class B scavenger receptor, CD36 [26-28]. Because CD36 is the major scavenger receptor involved in the recognition and internalization of mildly oxLDL [29], its increased expression and activity may play a major role in the development and progression of atherosclerosis. The present study demonstrates that differentiation of freshly isolated human monocytes into macrophages in the presence of gliclazide, a sulfonylurea with antioxidant properties [8-11], decreases CD36 expression, both at the mRNA and protein levels, as compared with untreated cells. This effect was also observed after incubation of human monocytes with NAC. In contrast, differentiation of human monocytes in the presence of vitamin E decreased CD36 mRNA expression, but failed to affect CD36 protein expression. The latter findings are in line with previous data demonstrating that CD36 expression is tightly regulated both at the transcriptional and posttranscriptional levels [30]. Because oxidative stress is involved in the regulation of CD36 expression [31], our results demonstrating that glyburide, a sulfonylurea without antioxidant properties, had no effect on macrophage CD36 expression suggest that the antioxidant attributes of gliclazide may account for its inhibitory effect on CD36 expression. Our observation that incubation of human macrophages with gliclazide inhibits advanced glycation end products–induced intracellular reactive oxygen species accumulation in these cells supports this possibility (data not shown). Our results also demonstrate that incubation of monocyte-derived macrophages in the presence of gliclazide or NAC decreased the uptake of mmLDL by human monocyte-derived macrophages. Whereas Devaraj et al [27] reported that vitamin E decreases CD36 activity in monocyte-derived macrophages as assessed by DiI-labeled oxLDL, we were unable to document an effect of this compound on mmLDL uptake by these cells. This apparent discrepancy in results may be related to differences in the experimental conditions used in the 2 studies. Specifically, use of fully copper-induced oxLDL and shorter length of incubation of the cells with vitamin E in Devaraj's study may underlie this discrepancy. To the best of our knowledge, our present results demon-

strate for the first time that NAC inhibits mmLDL uptake by human monocyte-derived macrophages.

In agreement with previous studies [27,29], we found that incubation of monocyte-derived macrophages with mmLDL results in foam cell formation. Our data demonstrate that gliclazide, despite decreasing macrophage CD36 activity, failed to reduce this event. This lack of effect of gliclazide on macrophage foam cell formation may be explained by the fact that decreased CD36 activity induced by the drug was not sufficient to reduce this biologic event. This possibility is supported by our observation that NAC, which more effectively reduces mmLDL uptake by macrophages than gliclazide, under our experimental conditions, significantly inhibited foam cell formation. Studies conducted in macrophages cell lines and mouse peritoneal macrophages have suggested that vitamin E may prevent foam cell formation [32,33]. In contrast, no effect of vitamin E supplementation of human macrophages on the formation of foam cells was reported by Asmis and Jelk [34]. In agreement with results from this study, we found that differentiation of human monocytes into macrophages in medium supplemented with vitamin E does not affect foam cell formation. Discrepancy in results concerning the effect of vitamin E on foam cell formation may derive from the use of different cell types, that is, human macrophages vs mouse peritoneal macrophages or macrophage cell lines, those ones being especially considered as poor models of human foam cell formation because of their high proliferation rates and very low vitamin E levels [33].

Increased sensitivity to LPS has been demonstrated during the process of monocyte differentiation into macrophages [20]. Accumulation of the redox-sensitive transcription factor nuclear factor κB during maturation of monocytes to macrophages has been recently suggested to prime the cells for such enhanced responsiveness to LPS [35]. Our data demonstrate that addition of gliclazide throughout monocyte maturation to macrophages decreases LPS-induced secretion of the proatherogenic cytokines TNF-α and IL-6. These findings are in line with previous results demonstrating that gliclazide decreases LPS-induced TNF-α secretion by human peripheral blood mononuclear cells [36] and monocytes from diabetic patients [37]. In contrast to previous studies conducted in rat Kupffer cells [38] and murine alveolar macrophages [39], we did not document any effect of vitamin E on TNF-α and IL-6 production by LPS-stimulated human macrophages. Use of different cell types may account for these contrasting observations. Previous studies have demonstrated that glyburide inhibits LPS-induced cytokine production by murine macrophages through blockade of ATP-binding cassette (ABC) proteins [40,41]. Whether this mechanism is implicated in the inhibitory effect of glyburide on LPS-induced cytokine production reported in the present study remains to be determined. Furthermore, the possibility that gliclazide decreases LPS-induced TNF-α and IL-6 secretion through inhibition of ABC proteins cannot be ruled out and warrants further studies. Our data showing that gliclazide inhibits LPS-induced TNF-α and IL-6 secretion in different manners suggest that the drug may exhibit a differential immunomodulating effect on macrophage secretory and cellular activities, as previously demonstrated for some pharmacologic compound [42,43]. Of note, our findings that gliclazide decreases both LPS-induced TNF-α and IL-6 secretion at gliclazide concentrations of 10 μg/mL, which is in the range of plasma therapeutic concentrations (5-10 μg/mL), suggest that this effect may be of clinical relevance in diabetic patients.

In conclusion, our results demonstrate that gliclazide, at therapeutically effective concentrations, reduces monocyte-derived macrophage CD36 expression and activity, although it did not affect foam cell formation. Gliclazide also reduces macrophage activation, as evidenced by decreased LPS-induced TNF-α and IL-6 secretion. Overall, our data indicate that gliclazide may regulate some key biologic events associated with the process of monocyte differentiation into macrophages. These results along with previous clinical and experimental data showing that gliclazide has antiatherogenic effects [44-47] suggest that this drug may help prevent or reduce macrovascular complications associated with type 2 diabetes mellitus.

Acknowledgment

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