α-Tocopherol decreases CD36 expression in human monocyte-derived macrophages

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Abstract **Cholesterol-laden macrophages are the hallmark of atherogenesis. The class B scavenger receptor, CD36, binds oxidized low density lipoprotein (OxLDL), is found in atherosclerotic lesions, and is upregulated by OxLDL. We tested the effects of α-tocopherol (AT) enrichment of human monocyte-derived macrophages on CD36 expression and cholesteryl ester accumulation. Monocytes isolated from normal volunteers were cultured into macrophages. Macrophages were enriched overnight with various doses of AT (25, 50, and 100 μM). LDL from normal volunteers was oxidized or acetylated (AcLDL) and incubated with macrophages for 48 h at a concentration of 50 or 100 μg/ml. CD36 expression was assessed by flow cytometry. Quantitative analysis of scavenger receptor class A (SR-A) activity was performed with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanide perchlorate (DiI)-labeled LDL. CD36 expression was maximal after 8–10 days of culture. AT (≥50 μM) significantly decreased CD36 expression upregulated by OxLDL and AcLDL ($P < 0.01$). Other antioxidants (β- or γ-tocopherol) or protein kinase C inhibitors failed to decrease CD36 expression. Concomitantly, DiI-AcLDL and DiI-OxLDL uptake was significantly decreased after AT treatment ($P < 0.001$). Cholesteryl ester accumulation was significantly decreased after AT enrichment (AcLDL + AT, 77% inhibition; OxLDL + AT, 42% inhibition). In conclusion, AT decreases both CD36 and SR-A expression and cholesteryl ester accumulation in human macrophages. This provides additional scientific support for the antiatherogenic properties of AT.**—Devaraj, S., I. Hugou, and I. Jialal. **α-Tocopherol decreases CD36 expression in human monocyte-derived macrophages.** ***J. Lipid Res.*** **2001.** 42: **521–527.**

Supplementary key words atherosclerosis • scavenger receptor • LDL • antioxidant • vitamin E

A number of epidemiological studies have demonstrated that increased levels of low density lipoproteins (LDL) are associated with an increased risk of atherosclerosis. However, the mechanism(s) by which LDL promotes the evolution of the fatty streak lesion remains to be elucidated. Uptake of cholesterol by the classic LDL receptor pathway cannot result in appreciable cholesterol accumulation because the LDL receptor is subject to feedback inhibition by the intracellular cholesterol content (1). However, modified LDL, such as acetylated or oxidized LDL (AcLDL and OxLDL, respectively), can be taken up by the scavenger receptor (SR) pathway, resulting in unregulated cholesterol accumulation and foam cell formation. The most plausible and biologically relevant modification of LDL is oxidation (2–5). Several lines of evidence point to a proatherogenic role for OxLDL. All major cells of the artery wall, such as monocyte-derived macrophages, endothelial cells, and smooth muscle cells, can oxidatively modify LDL in vitro (2–5).

Modified LDL, for example, OxLDL, is processed via the SR pathway. Because these receptors are not regulated by the intracellular cholesterol content, they can lead to substantial cholesteryl ester accumulation (6). The first SR was identified on macrophages as an AcLDL receptor, SR class A or SR-A (7). However acetylation does not occur under physiological circumstances. Other SR include MARCO, macrosialin, LOX-1, and SREC (8).

A second class of SR, SR class B (SR-B), has been identified as the OxLDL receptor. CD36, first cloned by Endemann et al. (9), is a member of the SR-B family. It is an 88-kDa transmembrane glycoprotein expressed on monocyte-derived macrophages, platelets, adipocytes, and certain endothelial and epithelial cells (10). CD36 may play a quantitatively significant role in modified LDL binding to macrophages (9). In addition to binding AcLDL and OxLDL (11), CD36 binds thrombospondin (12), anionic phospholipids (13), and long-chain fatty acids and collagen (14). CD36 is highly expressed on lipid-laden macrophages in human atherosclerotic aorta (15). Monocyte-derived macrophages obtained from patients with inherited deficiency of CD36 bind 40% less OxLDL and accumulate 40% less cholesteryl ester compared with control cells. In addition, elicited macrophages derived from CD36 knock-

Abbreviations: AT, α-tocopherol; OxLDL, oxidized low density lipoprotein; SR, scavenger receptor.

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out mice bound and internalized 60% less modified LDL (16–18). CD36 and apolipoprotein E (apoE) double-knockout mice exhibit significantly less atherosclerosis than do apoE$^{-/-}$ mice (18). CD36 has been shown to be upregulated by OxLDL in HL-60 and THP-1 cells via induction of the peroxisome proliferator-activated receptor γ (PPARγ) (19) and in smooth muscle cells and RAW cells via protein kinase C (PKC) (20). These findings suggest that CD36 plays a key role in foam cell formation in atherosclerotic lesions and is therefore an important receptor to modulate. Also, it appears that the majority of modified LDL processing by macrophages is via CD36 and SR class A (SR-A) (40–90%) (16, 18, 20a).

α-Tocopherol (AT) is the most potent member of the vitamin E family and is a potent lipophilic chain-breaking antioxidant (21). Decreased intakes are associated with increased risk of atherosclerosis, whereas increased intakes of AT appear to be cardioprotective. In addition to decreasing the oxidative susceptibility of LDL, AT has been shown to have direct antiatherogenic effects on endothelial cells, monocytes, and smooth muscle cells (22). The monocyte-derived macrophage is a readily accessible cell and is a critical cell that is present in all stages of atherogenesis. Although AT has been shown to reduce cholesterol esterification and uptake of AcLDL in J774 cells, there is a paucity of data examining the role of AT in CD36 expression and activity in human monocyte-derived macrophages, the pivotal cell in early atherogenesis. Thus, the aim of this study was to determine the effect of AT on CD36 expression and function in human monocyte-derived macrophages.

MATERIALS AND METHODS

Human monocyte-derived macrophages

Mononuclear cells were isolated from human volunteers by density-gradient centrifugation, using Ficoll-Hypaque (Sigma, St. Louis, MO) and were suspended in RPMI 1640 plus antibiotics (50 μg/ml each of penicillin and streptomycin) as described previously (23). Mononuclear cells were plated in six-well plates (Primaria; Becton Dickinson, Franklin Lakes, NJ) and incubated for 2 h at 37°C. Nonadherent cells were then removed by washing the wells twice with RPMI, and the remaining adherent cells were grown in the culture medium supplemented with 10% autologous human serum and 2 mM glutamine. The medium was replaced every 2–3 days. The cells were used after 8 days of culture.

Cell viability, determined by trypan blue exclusion, was >94% in all experiments. (*RRR*)-AT was dissolved in dimethyl sulfoxide (DMSO), and macrophages were incubated in the presence of vehicle control or AT for 24 h before addition of modified LDL for 48 h.

Isolation and modification of LDL

LDL (1.019–1.063 g/ml) was isolated from ethylenediaminetetraacetic acid (EDTA)-plasma of fasting healthy volunteers by sequential ultracentrifugation, desalted through columns (Econopak DG; Bio-Rad, Hercules, CA), and sterilized by filtration through a 0.22-μm pore size filter (Millipore, Waltham, MA), and stored under nitrogen in the dark at 4°C. Protein content was determined by the method of Lowry et al. as described previously (23).

LDL was labeled with the fluorescent probe 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanide perchlorate (DiI; Molecular Probes, Eugene, OR), as described by Innerarity, Pitas, and Mahley (24) and acetylated according to the procedure of Basu et al. as described previously (25). Briefly, LDL was incubated overnight at 37°C with 50 μl of DiI in DMSO (3 mg/ml) for each milligram of LDL protein. The labeled LDL was then reisolated by ultracentrifugation (24). The LDL must be labeled before any other modification such as acetylation or oxidation.

OxLDL was prepared by incubating LDL with 5 μM $CuSO_4$ for 8 h at 37°C as described previously (26). The extent of oxidative modification of LDL was evaluated by examining relative electrophoretic mobility in an agarose gel and by quantitation of conjugated dienes at 234 nm as described previously (26). We have measured the endotoxin concentration in LDL, using reagents from BioWhittaker (Walkersville, MD), as described previously (27); the concentration was <60 pg/ml, a level devoid of any biological activity.

DiI-LDL uptake by macrophages

After an overnight incubation with AT (25–100 μM), macrophages were incubated with DiI-AcLDL (50 μg/ml) for 48 h. After incubation with DiI-AcLDL, cells were washed with phosphate-buffered saline (PBS) and 1 ml of lysis reagent was added (0.1% sodium dodecyl sulfate in 0.1 N NaOH) (24). The fluorescence intensity of the lysate was measured on microtiter plates, with excitation and emission wavelengths set at 520 and 580 nm, respectively. Results were expressed as mean fluorescence intensity (MFI) per 10^5 cells. In another set of experiments, after incubation with DiI-AcLDL, cells were washed with PBS and analyzed for DiI-LDL uptake by flow cytometry.

Flow cytometry analysis of CD36 expression

Cells were washed with PBS and detached with 5 mM EDTA in PBS, pH 8.2. The pellet was incubated with fluorochrome-conjugated antibody for 30 min on ice. The monoclonal antibodies used were CD36-fluorescein isothiocyanate (FITC), CD14-TC, and isotype controls. The cells were then washed to remove the unspecific bound antibody and fixed with 1% paraformaldehyde. Isotype-matched phycoerythrin, FITC, and Tricolor (TC) controls were run with each sample. The cells were analyzed on a Becton Dickinson FACScan, using CellQuest software as reported previously (28).

Cholesteryl ester levels in human macrophages

After incubation with AcLDL or OxLDL in the presence or absence of AT or blocking antibody, cells were extracted with hexane–isopropanol 3:2 (v/v) and total and free cholesterol levels were measured by gas chromatography after extraction, as described previously (25). Esterified cholesterol levels were obtained by subtracting free cholesterol from TC.

Isolation of total RNA and reverse transcription-polymerase chain reaction (RT-PCR)

Cells were lysed in RNAzol (GIBCO-BRL, Gaithersburg, MD); total cellular RNA was extracted with chloroform and precipitated in isopropanol. CD36 mRNA expression was performed with RT-PCR reagents from Ambion (Austin, TX) with SuperTaq polymerase and specific primers for CD36 (Integrated DNA Technologies, Coralville, IA) for 30 cycles at 94°C, 30 s; 55°C, 30 s; 72°C, 30 s; and, finally, at 72°C, 5 min, using 18S RNA as internal standard. Primers that were used to identify CD36 matched published sequences 5′-CAGCCCAATGGAGCCATC-3′ and 5′-CAGCGTAGATA GACCT GC-3′ as described previously (29). The amplified transcripts were analyzed by loading on 2% agarose gels, and the signal intensity of the bands with the expected size (487 bp) was examined.

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Statistical analyses

Paired *t*-tests were used to assess significant differences in parameters measured in the presence and absence of AT and the level of significance was set at $P < 0.05$. All experiments have been performed at least three times.

RESULTS

After incubation of human monocyte-derived macrophages overnight with 25, 50, and 100 μM AT, there was a dose-dependent enrichment of macrophages with AT (1.5-, 2-, and 2.3-fold, respectively; n = 3 experiments).

CD36 expression was time dependent and was maximal at 8–12 days of culture (**Fig. 1**; n = 4 experiments). AT enrichment resulted in significant inhibition of CD36 expression on days 4, 8, and 12. All subsequent experiments were conducted after 8 days in culture. In contrast to AT, incubation with β- or γ-tocopherol (100 μM), while resulting in a 1.7- and 2.4-fold enrichment in macrophages compared with control, did not significantly affect CD36 expression (16% and 8% decrease compared with control, $P = 0.11$ and 0.37, respectively; n = 4 experiments).

Incubation with AT (≥50 μM) significantly decreased macrophage CD36 expression after incubation with AcLDL (46% and 71% with AT at 50 and 100 μM, respectively) and OxLDL (47% and 77% with AT at 50 and 100 μM, respectively) (**Fig. 2A** and **B**; n = 6). Also, incubation of macrophages with the blocking antibody to CD36 inhibited its expression induced by both AcLDL and OxLDL (88%).

DiI-AcLDL and DiI-OxLDL uptake was studied to determine the effect of AT on SR-A activity. DiI-AcLDL uptake was significantly inhibited after incubation with fucoidan (**Fig. 3A** and **B**, n = 4, $P < 0.01$). AT significantly inhibited DiI-AcLDL as well as DiI-OxLDL uptake by human macrophages (Figs. 3A and B). Furthermore, the combination of fucoidan and AT was additive with regard to inhibition of DiI-AcLDL and DiI-OxLDL uptake (AcLDL: 128 ± 14.9 MFI/10^5 cells; AcLDL + fucoidan, 50 μg/ml: 68 ± 11.8 MFI/10^5 cells; AcLDL + AT: 87 ± 9 MFI/10^5 cells; AcLDL + AT + fucoidan: 44 ± 8 MFI/10^5 cells; OxLDL: 118 ± 12 MFI/10^5 cells; OxLDL + fucoidan: 72 ± 15 MFI/10^5 cells; OxLDL + AT: 83 ± 11 MFI/10^5 cells; OxLDL + AT + fucoidan: 49 ± 8 MFI/10^5 cells). Similar results were obtained with polyinosinic acid (100 μg/ml).

Incubation of macrophages with AcLDL or OxLDL significantly augmented cholesteryl ester accumulation in macrophages compared with control (**Fig. 4**; n = 4). As is depicted in Fig. 4, after AT enrichment, there was a significant decrease in cholesteryl ester accumulation in macrophages compared with AcLDL alone (77%, $P < 0.001$) or OxLDL alone (48%, $P < 0.005$).

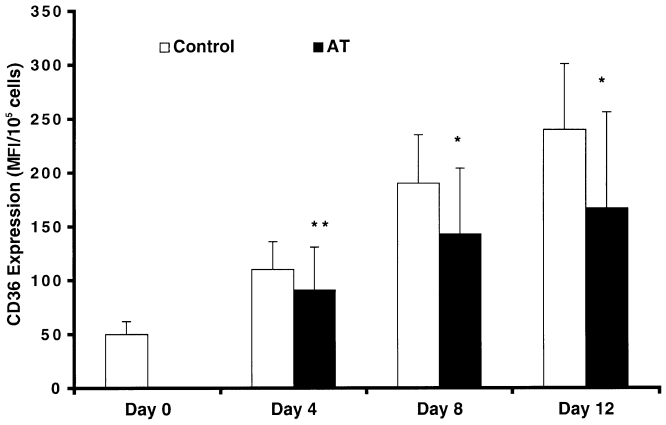

Fig. 1. Time course of CD36 expression. Monocytes were isolated from the peripheral blood of healthy human volunteers and cultured for 12 days in autologous serum to differentiate into macrophages, enriched with AT (100 μM; solid columns) or vehicle control (open columns) for 24 h before assessment of CD36 expression by flow cytometry as described in Materials and Methods. Data are expressed as means ± SD of four experiments. * $P < 0.001$ and ** $P < 0.01$ compared with control.

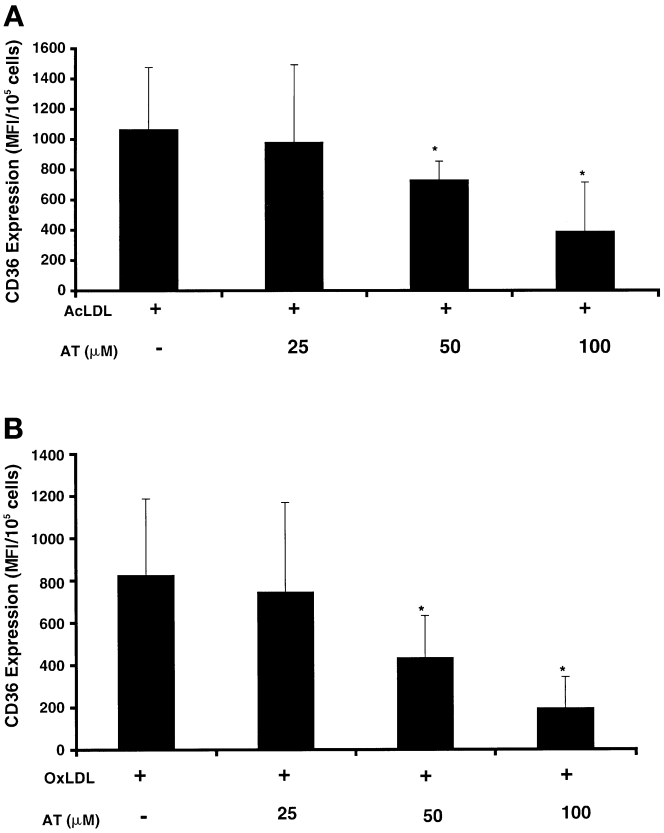

Fig. 2. A: Effect of AT enrichment of macrophages on AcLDL-induced CD36 expression. Human monocyte-derived macrophages were incubated in the presence of vehicle control or AT (25, 50, or 100 μM) for 24 h followed by addition of AcLDL, 50 μg/ml, for 48 h; CD36 expression was assessed by flow cytometry as described in Materials and Methods. Data are expressed as means ± SD of six experiments. * $P < 0.02$ compared with AcLDL. B: Effect of AT enrichment of macrophages on OxLDL-induced CD36 expression. Human monocyte-derived macrophages were incubated in the presence of vehicle control or AT (25, 50, or 100 μM) for 24 h followed by addition of OxLDL, 50 μg/ml, for 48 h; CD36 expression was assessed by flow cytometry as described in Materials and Methods. Data are expressed as means ± SD of six experiments. * $P < 0.02$ compared with OxLDL.

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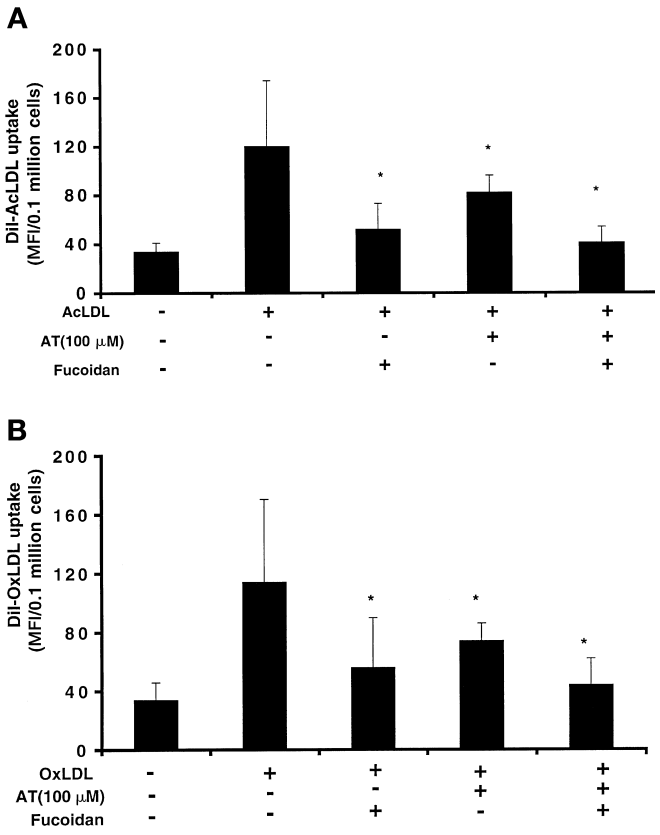

Fig. 3. A: Effect of AT enrichment of macrophages on DiI-AcLDL uptake by human macrophages. Human monocyte-derived macrophages were incubated in the presence of vehicle control or AT (100 μM) for 24 h followed by addition of AcLDL, 50 μg/ml, for 48 h in the presence and absence of fucoidan, 50 μg/ml; DiI-AcLDL uptake was assessed by flow cytometry as described in Materials and Methods. Data are expressed as means ± SD of four experiments. * $P < 0.01$ compared with AcLDL. B: Effect of AT enrichment of macrophages on DiI-OxLDL uptake by human macrophages. Human monocyte-derived macrophages were incubated in the presence of vehicle control or AT (100 μM) for 24 h followed by addition of OxLDL, 50 μg/ml, for 48 h in the presence and absence of fucoidan, 50 μg/ml; DiI-OxLDL uptake was assessed by flow cytometry as described in Materials and Methods. Data are expressed as means ± SD of four experiments. * $P < 0.01$ compared with OxLDL.

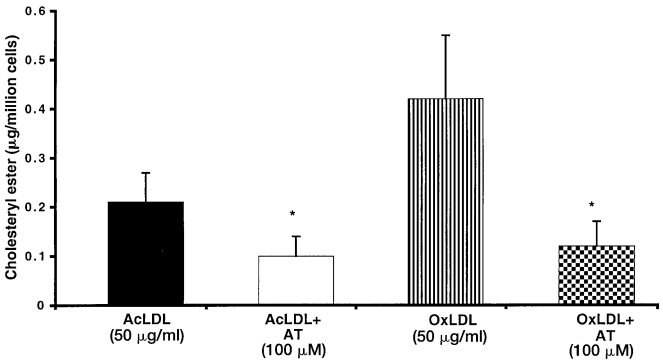

Fig. 4. Effect of AT enrichment of macrophages on cholesteryl ester accumulation. Human monocyte-derived macrophages were incubated in the presence of vehicle control or AT (100 μM) for 24 h followed by addition of AcLDL/OxLDL, 50 μg/ml, for 48 h; cholesteryl ester levels were measured as described in Materials and Methods. Data are expressed as means ± SD of four experiments. * $P < 0.005$ compared with AcLDL/OxLDL.

CD36 mRNA expression was upregulated in the presence of AcLDL or OxLDL. However, incubation of macrophages with AT had no effect on CD36 mRNA in control cells or in AcLDL- or OxLDL-loaded cells (**Fig. 5**, n = 3 experiments). There was no significant difference in CD36/18S mRNA ratio in the absence and presence of AT.

To examine mechanisms by which AT decreased CD36 expression, the effect of PKC inhibitors on CD36 expression was also examined by flow cytometry, because AT has been shown to inhibit PKC. Calphostin C (250 and 500 nM), bisindoleylmaleimide (0.1 and 1 μM), and chelerythrine (3 μM) did not result in any significant reduction in CD36 expression (n = 4 experiments; data not shown).

DISCUSSION

Low levels of AT have been associated with increased prevalence of coronary artery disease morbidity and mortality, and higher intakes of AT are associated with decreased incidence of cardiovascular disease (30–33). Oxidative modification of LDL is believed to be an important event in atherogenesis (2–5), and several studies have reported on the antioxidant effect of AT, that is, decreasing the susceptibility of LDL to oxidation (21, 34–36). Also, evidence of the antiatherogenic effects of AT on crucial cells in atherogenesis such as endothelial cells, platelets, smooth muscle cells, and monocyte-derived macrophages is mounting (21, 22). The monocyte-derived macrophage is a critical cell in the arterial intima and is present during all stages of atherogenesis. The formation of foam cells in the intima, the fatty streak lesion, is an important early event in atherogenesis (37). We have shown that high-dose AT supplementation (1,200 IU/day) significantly decreased monocyte proatherogenic activity (superoxide anion release; lipid oxidation; release of cytokines such as interleukin 1β, tumor necrosis factor α, and interleukin 6; and adhesion to endothelium) and decreased levels of sol-

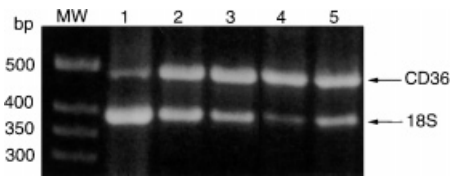

Fig. 5. Effect of AT enrichment of macrophages on CD36 mRNA. Human monocyte-derived macrophages were incubated in the presence of vehicle control or AT (100 μM) for 24 h followed by addition of AcLDL, 50 μg/ml, for 48 h; CD36 mRNA was quantitated by RT-PCR, using 18S RNA as control as described in Materials and Methods. Data are representative of three experiments. Lane 1, vehicle control; lane 2, AcLDL (50 μg/ml); lane 3, AcLDL + AT (100 μM); lane 4, OxLDL (50 μg/ml); lane 5, OxLDL + AT (100 μM).

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uble cell adhesion molecules in healthy human volunteers as well as in type 2 diabetic subjects, who are prone to accelerated atherosclerosis (23, 38). Further studies directed at mechanisms revealed that AT decreased monocyte superoxide release and lipid oxidation via inhibition of PKC activity (23), inhibited the release of interleukin 1β from human monocytes via inhibition of 5-lipoxygenase (39), decreased monocyte-endothelial cell adhesion via inhibition of nuclear transcription factor κB activity, and decreased expression of counterreceptors CD11b and very late antigen 4 (VLA-4) on monocytes (28). In addition to these intracellular effects, we now report that in human monocyte-derived macrophages, AT exerts novel effects of decreasing CD36 SR expression and subsequent cholesteryl ester accumulation, and could thereby reduce foam cell formation, a hallmark of the early atherosclerotic lesion.

CD36 is the major receptor responsible for the uptake of modified LDL. Human macrophage CD36 expression is upregulated by day 4 and is elevated about 5-fold in 8–10 days of culture. Nakagawa et al. (40) also reported that in human macrophages, CD36 expression was maximal after 10 days of culture. This is in contrast to Huh et al. (20a), who have previously shown that CD36 expression is upregulated and is maximal on day 4 (8- to 10-fold) and is reduced by day 8 of culture. This discrepancy may be because their monocytes were cultured in 5% AB serum, purchased from Sigma, in 24-well plates, whereas we used 10% autologous heat-inactivated serum for culturing our macrophages in 6-well plates. Furthermore, loading of macrophages with AcLDL or OxLDL significantly increases CD36 expression about 10-fold. This is in accordance with Han et al. (41, 42), who have shown that modified LDL and cellular cholesterol upregulate CD36 expression in J774.1 murine macrophages. Incubation of macrophages with AT at doses that can be achieved with supplementation significantly decreased CD36 expression in human monocyte-derived macrophages. To determine whether the inhibition by AT was due to its antioxidant properties, macrophages were incubated with β- or γ-tocopherol, which have comparable antioxidant potential and differ only in the position of the methyl groups on the chroman ring. CD36 expression in human monocyte-derived macrophages was not affected by these tocopherol homologs. Teupser, Thiery, and Seidel (43) have previously shown that α- and β-tocopherol, but not γ- or δ-tocopherol, downregulated SR-A activity in rabbit peritoneal macrophages and only α-tocopherol reduced AP-1 activity. However, they did not report on CD36 expression or cholesteryl ester accumulation. While this article was in preparation, Ricciarelli, Zingg, and Azzi (44) reported that α-tocopherol, but not β-tocopherol, reduced CD36 expression in a cultured human aortic smooth muscle cell line by downregulating CD36 mRNA and protein expression. However, they did not examine the effect of AT on SR-A activity or intracellular cholesteryl ester accumulation. Furthermore, they did not examine the effect of AT on CD36 expression in human monocyte-derived macrophages, the critical cells in atherogenesis, and did not test the effect of AT on CD36 mRNA in HL-60 cells. Also, the effect of AT on CD36 expression upregulated by modified LDL was not examined.

To examine whether AT only decreased CD36 expression or had effects on SR-A activity as well, quantitative analysis of SR activity was performed with DiI-labeled AcLDL and DiI-labeled OxLDL. DiI-AcLDL uptake was significantly inhibited after incubation with fucoidan or polyinosinic acid. AT significantly inhibited DiI-AcLDL as well as DiI-OxLDL uptake by human macrophages. Furthermore, the combination of fucoidan and AT was additive with regard to inhibition of DiI-AcLDL uptake. Thus, it appears that AT exerts dual effects in inhibiting SR-A and CD36 activities. Ricciarelli, Zingg, and Azzi (44) also demonstrated decreased uptake of Di-OxLDL by the human aortic smooth muscle cell line; however, they failed to assess whether this affected subsequent intracellular cholesteryl ester accumulation.

To elucidate whether decreased expression of CD36 translates to decreased cholesteryl ester accumulation, cholesteryl ester accumulation in macrophages was determined. Incubation with AcLDL or OxLDL significantly increased cholesteryl ester accumulation. AT significantly inhibited cholesteryl ester accumulation after incubation with AcLDL and OxLDL. Suzukawa et al. (45) reported that enrichment of J774, a murine macrophage cell line, with AT inhibited macrophage-mediated LDL oxidation and cholesteryl ester formation. However, they did not examine whether these effects of AT were due to the decreased capacity of macrophages to oxidize LDL or due to its effects on cholesterol metabolism. Asmis et al. (46) reported that enrichment of P:388D murine macrophages with AT reduced the cellular ratio of cholesteryl ester to free cholesterol after incubation with fetal bovine serum but not modified LDL. Shige et al. (47) reported that AT (50 μM) significantly reduced uptake of labeled AcLDL with a trend toward suppression of ACAT activity, resulting in decreased cholesteryl esterification in J774 cells, a murine macrophage cell line. However, they did not study the effect of AT on OxLDL-induced cholesteryl ester accumulation. The present study goes further in demonstrating that in human monocyte-derived macrophages, AT decreases CD36 expression and SR activity, and this is associated with decreased cholesteryl ester accumulation.

Finally, we examined whether the reduction in CD36 expression and activity in human monocyte-derived macrophages by AT was due to decreased message for CD36. Whereas CD36 mRNA expression was upregulated in the presence of both AcLDL and OxLDL as reported previously, AT enrichment did not have any effect on CD36 mRNA. In a cell line of smooth muscle cells, AT appears to downregulate CD36 mRNA (44). However, the difference in cell type used in their studies may underlie this discrepancy in findings. Thus, in human monocyte-derived macrophages, AT appears to downregulate CD36 expression at the posttranscriptional level. Feng et al. (20) have shown that induction of CD36 expression by OxLDL in a murine macrophage cell line, RAW 264.7, involves initial activation of PKC. AT has been shown to decrease PKC

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activity in monocytes (23). Thus, we tested the effect of PKC inhibitors on OxLDL-induced CD36 expression by flow cytometry. In our experiments with human monocyte-derived macrophages, three PKC inhibitors failed to significantly reduce CD36 expression. Thus, although AT decreases smooth muscle cell proliferation, platelet aggregation, and monocyte superoxide anion release and lipid oxidation via inhibition of PKC (21, 22), in two different cell systems (smooth muscle cells and human macrophages) CD36 expression is not regulated by AT via inhibition of PKC (44). It is possible that AT affects PPARγ, which has also been implicated in CD36 upregulation by OxLDL (19, 20) in macrophages. A decrease in the PPARγ pathway does not appear to be the mechanism involved because troglitazone, a PPAR agonist that comprises a vitamin E moiety, increases and does not decrease CD36 expression. Han et al. (48) showed that transforming growth factor β1 and β2 decreased CD36 expression and mRNA in THP-1 cells. Because we failed to show an effect on CD36 mRNA in human monocyte-derived macrophages, this is unlikely to explain the inhibition of CD36 expression and cholesteryl ester accumulation by AT. Future studies will be directed at elucidating the precise molecular mechanism by which AT decreases CD36 expression, such as its effect on tyrosine kinase activity and transforming growth factor β, which appear to regulate CD36 expression.

In summary, we report for the first time in human monocyte-derived macrophages that AT enrichment results in decreased CD36 expression, decreased SR activity, and decreased cholesteryl ester accumulation, and that the mechanism of inhibition appears to be posttranscriptional. These findings, along with other reported beneficial effects of AT (i. e., decrease in LDL oxidation, platelet aggregation, improvement in endothelial function, decrease in monocyte proatherogenicity, and smooth muscle cell proliferation) lend further support for the potential antiatherogenic effects of AT.

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