Am J Physiol Endocrinol Metab 293: E1820–E1827, 2007.
First published October 2, 2007; doi:10.1152/ajpendo.00315.2007.

Effects of lycopene on the induction of foam cell formation by modified LDL

Mariarosaria Napolitano,[1] Clara De Pascale,[2] Caroline Wheeler-Jones,[2] Kathleen M. Botham,[2] and Elena Bravo[1]

[1]*Department of Hematology, Oncology, and Molecular Medicine, Istituto Superiore di Sanitá, Rome, Italy; and* [2]*Department of Veterinary Basic Sciences, The Royal Veterinary College, London, United Kingdom*

Submitted 22 May 2007; accepted in final form 27 September 2007

Napolitano M, De Pascale C, Wheeler-Jones C, Botham KM, Bravo E. Effects of lycopene on the induction of foam cell formation by modified LDL. *Am J Physiol Endocrinol Metab* 293: E1820–E1827, 2007. First published October 2, 2007; doi:10.1152/ajpendo.00315.2007.—The effect of lycopene on macrophage foam cell formation induced by modified low-density lipoprotein (LDL) was studied. Human monocyte-derived macrophages (HMDM) were incubated with lycopene in the presence or absence of native LDL (nLDL) or LDL modified by oxidation (oxLDL), aggregation (aggLDL), or acetylation (acLDL). The cholesterol content, lipid synthesis, scavenger receptor activity, and the secretion of inflammatory [interleukin (IL)-1β and tumor necrosis factor (TNF)-α] and anti-inflammatory (IL-10) cytokines was determined. Lycopene was found to decrease the synthesis of cholesterol ester in incubations without LDL or with oxLDL while triacylglycerol synthesis was reduced in the presence of oxLDL and aggLDL. Scavenger receptor activity as assessed by the uptake of acLDL was decreased by ~30% by lycopene. In addition, lycopene inhibited IL-10 secretion by up to 74% regardless of the presence of nLDL or aggLDL but did not affect IL-1β or TNF-α release. Lycopene also reduced the relative abundance of mRNA transcripts for scavenger receptor A (SR-A) in THP-1 macrophages treated with aggLDL. These findings suggest that lycopene may reduce macrophage foam cell formation induced by modified LDL by decreasing lipid synthesis and downregulating the activity and expression of SR-A. However, these effects are accompanied by impaired secretion of the anti-inflammatory cytokine IL-10, suggesting that lycopene may also exert a concomitant proinflammatory effect.

low-density lipoprotein; scavenger receptors; cytokines; macrophage

LYCOPENE IS A PLANT CAROTENOID naturally present in tomatoes and tomato products. Many epidemiological studies have suggested that the high intake of fruit and vegetables associated with, for example, the Mediterranean diet reduces the risk of the development of atherosclerosis and related cardiovascular disease (CVD; see Ref. 20), and there is good evidence to suggest that micronutrients such as lycopene contribute to this beneficial effect (37, 47). Dietary intake of lycopene or tomatoes/tomato products has been shown to increase its levels in serum and adipose tissue and is inversely correlated with the incidence of CVD (37, 34). These observations have generated scientific interest in lycopene as a potential dietary preventative agent for heart disease.

Atherosclerosis begins with the development of fatty streaks, which are formed when macrophages that have invaded the artery wall scavenge lipid from plasma lipoproteins in the subendothelial space, eventually becoming so engorged that they form foam cells (22). It is well established that low-density lipoprotein (LDL) plays a major role in macrophage foam cell formation, but modification of the particles, either chemically or by oxidation or aggregation, processes that occur within the artery wall, is necessary before extensive lipid accumulation is induced. Uptake of native LDL via the LDL receptor (LDLr) is downregulated when intracellular cholesterol levels begin to rise and thus does not lead to foam cell formation (1). The modified LDLs, however, are taken up mainly by unregulated scavenger receptors such as scavenger receptor (SR) A and the class B receptor CD36, allowing large amounts of lipid to accumulate intracellularly (46).

Several studies have reported that serum or tissue lycopene levels are inversely related to intimal wall thickness or lesions in the carotid artery and aorta, suggesting that lycopene may protect against the development of atherosclerosis (3, 33, 37). Because lycopene is an efficient antioxidant (12), it has been proposed that this property may be responsible for its beneficial effects. In support of this, lycopene has been demonstrated to protect LDL from oxidation in vitro, and some dietary studies have shown that lycopene-containing foods increase resistance of LDL to oxidation in vivo (3, 46). On the other hand, plasma lycopene concentrations in smokers are not consistently lower than in nonsmokers (3) as might be expected, suggesting that other mechanisms in addition to its antioxidant activity may be involved in its protective effects. Other potential protective mechanisms include the inhibition of cholesterol synthesis and enhancement of LDL degradation (13).

Despite the important role played by oxidative stress in macrophage foam cell formation, few studies have addressed the effects of lycopene on this process. Our previous work has shown that incorporation of lycopene in chylomicron remnants, the physiological carrier of dietary lipids in the blood, enhanced their induction of lipid accumulation in macrophages derived from the human cell line THP-1. Similar results were also obtained with the antioxidant lipophilic drug probucol (24, 25). This unexpected and novel finding suggests that dietary antioxidants carried in chylomicron remnants may promote, rather than inhibit, macrophage foam cell formation. In addition, Furhman et al. (13) have reported that lycopene inhibits cholesterol synthesis and upregulates the activity of the LDLr in the murine macrophage cell line J774. Nothing is known, however, about how lycopene affects the response of human macrophages to modified LDL.

It is now well recognized that atherosclerosis is an inflammatory disease (40), and there is some evidence to suggest that the beneficial effects of lycopene may result from modulation of inflammatory responses. For example, plasma levels of

Address for reprint requests and other correspondence: E. Bravo, Istituto Superiore di Sanitá, Dept. of Haematology, Oncology and Molecular Medicine, Viale Regina Elena 299, 00161 Rome, Italy (e-mail: bravo@iss.it).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

lycopene have been found to be reduced in the acute phase response (17), and Riso et al. (35) have reported that concentrations of the proinflammatory cytokine tumor necrosis factor-α (TNF-α) in the blood of healthy volunteers were decreased after dietary supplementation with a tomato-based drink. However, although macrophages function as immune mediators and as important sources of chemotactic molecules and cytokines in atherosclerotic lesions during all stages of atherogenesis (40), the influence of lycopene on macrophage cytokine production has not been studied previously.

In this study, we have used human monocyte-derived macrophages (HMDM) and THP-1 macrophages to investigate the effects of lycopene on cholesterol accumulation, lipid synthesis, cytokine production, and the activity and expression of scavenger receptors in the presence or absence of native LDL or LDL modified by acetylation, oxidation, or aggregation. HMDM were used for most experiments, but, because these cells are obtained from individuals, they have the disadvantage that the interexperiment variation tends to be large. For this reason, the human cell line THP-1 was used for investigation of the effects of lycopene on the expression of mRNA for scavenger receptors.

MATERIALS AND METHODS

[9,10(n)^{3}H]oleic acid (9.2 Ci/mmol) and [4-^{14}C]cholesteryl oleate (60 mCi/mmol) were obtained from NEN Life Science Products (Boston, MA); [1α,2α(n)-^{3}H]cholesterol oleate (30–60 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Milan, Italy). Amplex Red Cholesterol Assay kit was purchased from Molecular Probes. SearchLight Proteome Arrays for Human Inflammatory Cytokine Array 3 were obtained from Endogen (TEMA ric, Bologna, Italy). Iscove's modified Dulbecco's medium (IMDM), FBS, Ficoll-Paque, penicillin, and streptomycin were supplied by Hyclone Europe CD14 MicroBeads, and LS separation columns were purchased from Miltenyi Biotec. Lycopene, fatty acid-free BSA, and commercial solvents were purchased from Sigma-Aldrich (Milan, Italy).

Isolation and modification of lipoproteins. The LDL (1.019–1.063 g/ml) fraction was isolated from pooled human plasma derived from fasted normolipidemic volunteers by sequential ultracentrifugation in a Beckman 60 Ti rotor (28), dialyzed against PBS at 4°C, sterilized by filtration through a 0.45-μm filter, stored at 4°C, and used within 10 days of isolation. Oxidized LDL (oxLDL) was prepared by incubating LDL with 10 μM $CuSO_4$ for 18 h at 37°C (15), and acetylated LDL (acLDL) was made by repeated addition of acetic anhydride to 4 mg/ml LDL diluted with saturated ammonium acetate at 4°C, as previously described (6, 28). On the day of experiment, an aliquot of native LDL (nLDL) was processed to obtain aggregated LDL (aggLDL) by vigorously vortexing nLDL for 1 min in a 12 × 75-mm polypropylene tube (4, 18). Guyton et al. (16) have demonstrated that vortexing LDL results in the formation of aggregates after as little as 5 s, and this is reflected in a rapid increase in absorbance at 680 nm, reaching a plateau of ~0.18 after 1 min. In close agreement with this, we found a rise in absorbance at 680 nm of 0.19 ± 0.03 (n = 3) after vortexing, indicating an increase in the size of the particles.

Isolation and culture of macrophages. Monocytes were isolated from human buffy coats by density gradient centrifugation as previously described (29), with some modifications. Buffy coats from the blood of healthy donors were diluted 1:3 (vol/vol) with PBS and layered on Ficoll-Paque. After centrifugation, white blood cells were collected and washed with PBS. CD14 MicroBeads were used for the positive selection of human monocytes from white blood cells; 300–400 × 10^6 total cells were applied to LS separation columns, and the total effluent was discarded. Monocytes (CD14-positive fraction) were flushed out, washed, transferred to 16-mm dishes at a density of 8 × 10^5 cells/ml, and cultured in IMDM containing 15% FBS (supplemented IMDM). The purity of isolated monocytes, monitored by flow cytometric analysis of CD14, ranged from 95 to 98%. The differentiation of monocytes to macrophages was monitored by the increased expression of CD71.

THP-1 monocytes were maintained in suspension in RPMI 1640 culture medium containing 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 50 μM β-mercaptoethanol at a density of 3–9 × 10^5 cells/ml at 37°C in 5% air-95% CO_2. The cells were induced to differentiate into macrophages by incubation with phorbol 12-myristate, 13-acetate (PMA, 200 ng/ml) for 72 h. After this time, cells adhering to the culture dishes were washed with warm PBS (3 × 2 ml) to remove any undifferentiated cells and traces of PMA.

Lycopene was dissolved in dimethyl sulfoxide (DMSO) under nitrogen, protected from light, and stored in aliquots at −80°. Medium containing lycopene was freshly prepared for each experiment and added to the cells 9–10 days after plating at a final concentration of 10 μM. Control incubations were performed with DMSO alone, with the concentration never exceeding 0.1% (vol/vol).

Determination of cell cholesterol content. HMDM cultured for 9 day were pretreated for 24 h with 10 μM lycopene or DMSO alone in supplemented IMDM. The cells were then washed and incubated for a further 24 h with lycopene in the presence or absence of nLDL, acLDL, oxLDL, or aggLDL (100 μg protein/ml) in serum-free IMDM. After incubation, the cells were washed with PBS and lysed by the addition of 0.5 M phosphate buffer containing 0.25 M NaCl, 25 mM cholic acid, and 0.5% Triton X-100. Total and unesterified cholesterol (UC) in the cell lysates was determined using the Amplex red cholesterol assay according to the manufacturer's instructions (2).

Determination of cholesterol and triacylglycerol synthesis. The effects of lycopene on cholesterol esterification and triacylglycerol (TG) synthesis were evaluated by measuring the incorporation of [^{3}H]oleic acid in cholesteryl ester (CE) or TG, as previously described (28). After plating (10 days), HMDM were incubated with supplemented IMDM containing lycopene (10 μM). After 24 h, the cells were washed two times with PBS without Ca^{2+} and Mg^{2+} and incubated for 6 h in serum-free IMDM with or without nLDL, aggLDL, acLDL, or oxLDL (50 μg protein/ml) in the presence of 326 nM oleic acid and 6 μCi/ml [^{3}H]oleate bound to fatty acid-free BSA (2%). After incubation and washing, HMDM lipids were extracted with hexane-isopropyl alcohol (3:2, vol/vol). [^{14}C]CE was added as internal standard to estimate recovery. Lipids were separated by thin-layer chromatography on silica gel (Merck) with hexane-diethyl ether-acetic acid (70:30:1). The bands corresponding to CE and TG were identified by comparison with the appropriate standards and scraped in vials, and the radioactivity was determined by liquid scintillation counting (LS5000 Beckman) using Ready-gel (Beckman, Milan, Italy).

Determination of scavenger receptor activity. The effects lycopene on scavenger receptor activity in HMDM were evaluated by measuring the internalization of labeled acLDL ([^{3}H]CE-acLDL) or aggLDL ([^{3}H]CE-acLDL) (28). To prepare LDL labeled in the CE moiety, human plasma was adjusted to a density of 1.019 g/ml and centrifuged at 40,000 revolutions/min (rpm) for 18 h. The upper layer was discarded, and 100 μCi of [^{3}H]cholesterol were added drop by drop, with gentle stirring, to the lower phase. After incubation for 18 h at 37°C, the plasma was centrifuged (40,000 rpm for 18 h at 4°C) at density = 1.063 g/ml. The top fraction containing [^{3}H]CE-LDL was dialyzed and acetylated or aggregated as described above. Approximately 94% of the radiolabel was associated with CE and the remainder with UC.

Monocytes cultured for 10 days in supplemented IMDM were pretreated with either DMSO (control) or lycopene (10 μM) for 20 h, and the cells were then washed (3 times) with PBS and incubated for 24 h with [^{3}H]CE-acLDL [100 μg protein/ml; ~156,000 disintegrations/min (dpm)/well] or [^{3}H]CE-aggLDL (100 μg protein/ml; ~156,700 dpm/well), each in combination with either DMSO (con-

trol) or lycopene (10 μM). After incubation and washing with PBS containing Ca^{2+}, Mg^{2+}, and BSA (0.2% wt/vol) followed by PBS alone, lipids were extracted and processed as described above to determine the radioactivity associated with cellular cholesterol and CE. Total labeled cholesterol taken up by HMDM was calculated as the sum of the radioactivity associated with the [^{3}H]UC and [^{3}H]CE bands and expressed as picomoles [^{3}H]cholesterol per hour per milligram cell protein.

Determination of macrophage cytokine secretion. To study the effect of lycopene on cytokine secretion by human macrophages, HMDM cultured for 9 days were incubated in supplemented IMDM in the absence (DMSO only) or presence of 10 μM lycopene for 24 h at 37°C in 5% CO_2. After incubation, macrophages were washed two times with PBS and incubated for a further 24 h with or without nLDL or aggLDL (100 μg protein/ml). The medium was then collected, centrifuged at 12,000 rpm to remove debris, and stored at −80°C until analysis. Determination of TNF-α, interleukin (IL)-1β, and IL-10 concentrations was performed with SearchLight Proteome Arrays for Human Inflammatory Cytokine Array 3, according to the manufacturer's instructions. As a positive control, cells were incubated for 24 h with 1 μg/ml lipopolysaccharide.

mRNA analysis. THP-1 macrophages were incubated with lycopene (10 μM) or DMSO for 24 h. nLDL, acLDL, or aggLDL (100 μg protein/ml) was then added, and the incubations continued for a further 24 h. Total RNA was extracted from the cells using an RNAeasy Plus Mini Kit (Quiagen, Crawley, UK), and the abundance of transcripts for SR-A, SR-B1, and CD36 was determined by quantitative real-time PCR (qPCR). The reverse transcription reaction was carried out using an Omniscript RT kit (Quiagen) according to the manufacturer's instructions. cDNA was amplified using an Opticon 2 DNA Engine (MJ Research) using SYBR Green Jump Start Taq Ready Mix (Sigma, Gillingham, UK). The conditions were as follows: denaturation at 94°C for 2 min, followed by amplification (94°C, 15 s) and annealing [58°C for 1 min; glyceraldehyde-3-phosphate dehydrogenase (GAPDH)]; 60°C for 1 min (CD36, SR-A, SR-B1) and extension (72°C for 1 min) for 37 cycles; and finally a melting curve program (60–95°C, rate of 0.2°C/s). The threshold cycle values were determined by automated threshold analysis, using Opticon Monitor 2 software. GAPDH was used as the standard housekeeping gene. The primers employed and the product sizes are shown in Table 1. The relative quantification of mRNA expression was calculated as described by Pfaffl (32).

Other analytical methods. At the end of each experiment, HMDM were treated with 1 M NaOH for measurement of cellular protein by the method of Bradford (9) using BSA as standard.

Data are given as means ± SE of the experiments. Each experimental point was performed in duplicate. Data were analyzed by ANOVA followed by Bonferroni's multiple-comparison test post hoc, except where indicated otherwise.

RESULTS

Effects of lycopene on the cholesterol content of HMDM treated with nLDL or modified LDL. HMDM were pretreated for 24 h with or without lycopene (10 μM) and incubated for a further 24 h with lycopene in the presence or absence of nLDL, acLDL, oxLDL, or aggLDL (100 μg protein/ml). The cellular cholesterol content was then determined (Fig. 1). There were no significant differences in the amount of cholesterol accumulated in the cells in lycopene-treated compared with the control cells in any of the conditions tested. However, there was a tendency toward a decrease in cholesterol accumulation in the incubations containing lipoproteins (up to 26%). When these (nLDL, acLDL oxLDL, and aggLDL) were considered together, there was a significant reduction in cell cholesterol content in the presence of lycopene ($P < 0.05$, Student's paired *t*-test).

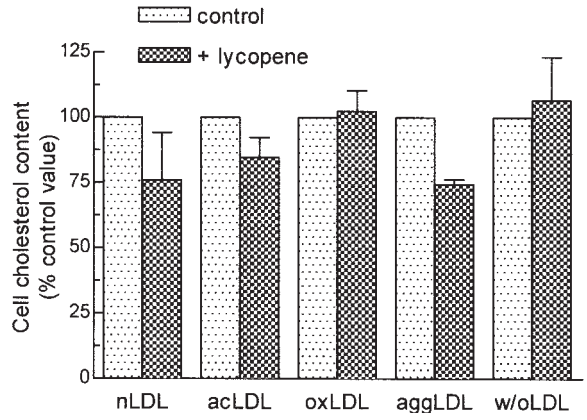

Fig. 1. Effects of lycopene on the cholesterol content of human monocyte-derived macrophages (HMDM) treated with native low-density lipoprotein (nLDL) or modified lipoprotein lipase (LDL). HMDM were pretreated for 24 h with 10 μM lycopene or DMSO alone and then washed and incubated for a further 24 h with lycopene or DMSO (control) in the presence or absence of nLDL or LDL modified by acetylation (acLDL), oxidation (oxLDL), or aggregation (aggLDL) (100 μg protein/ml). The cholesterol content of the cells was determined using the Amplex red cholesterol assay. Data are expressed as %control values and are means from 3 experiments in duplicate. Error bars show the SE.

Effects of lycopene on lipid synthesis in HMDM treated with nLDL or modified LDL. The synthesis of TG and cholesterol ester in HMDM exposed to native or modified LDL in the presence or absence of lycopene was determined. After 24 h incubation with lycopene, nLDL, acLDL, oxLDL, aggLDL, or DMSO only was added, and the incorporation of [^{3}H]oleate into TG and cholesterol ester in the following 6 h was measured. The results are shown in Fig. 2. Lycopene had no effect on TG synthesis in the absence of lipoprotein but tended to cause a decrease in the presence of all types of LDL. The changes were significant when oxLDL or aggLDL were used ($P < 0.05$) and were generally more marked with modified LDLs (−22 to 35%) than with nLDL (−15%; Fig. 2*A*).

Table 1. *Primer sequences and product sizes for determination of scavenger receptor mRNA expression*

Gene	Primer Sequence		Product Size, bp
	Sense	Antisense	
SR-B1	TTCTACACTCAGCTGGTGTTGATG	AGCGCCAGGAGGAGCTACT	65
CD36	CTCTTTCCTGCAGCCCAATG	GCTGCAGAAGAATGTCATTAAATCTT	75
SR-A	GCCAACCTCATGGACACAGA	GCTGCAGAAGAATGTCATTAAATCTT	79
GAPDH	CAACGGATTTGGTCGTATTGG	GCAACAATATCCACTTTACCAGAGTTAA	72

SR, scavenger receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

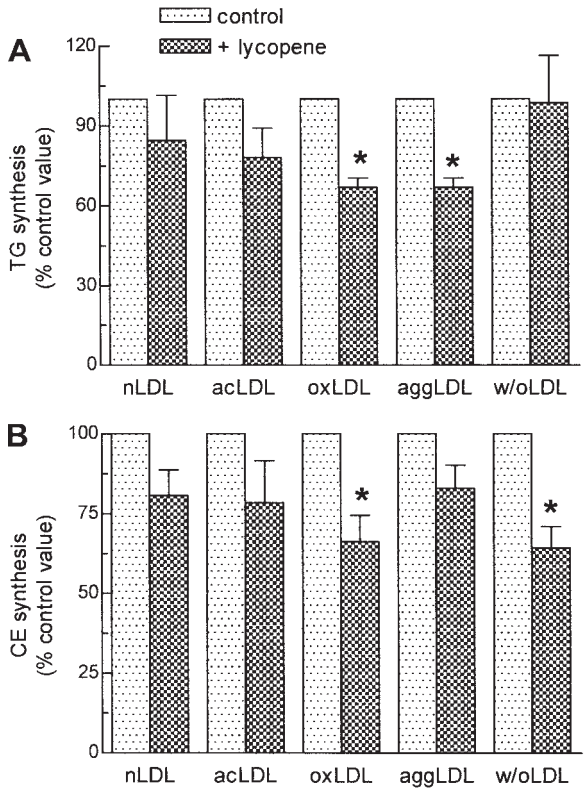

Fig. 2. Effects of lycopene on lipid synthesis in HMDM treated with nLDL or modified LDL. HMDM were pretreated with lycopene (10 μM) or DMSO for 24 h, the cells were then washed, and the incubation was continued for 6 h with lycopene or DMSO (control) in the presence or absence of nLDL, aggLDL, acLDL, or oxLDL (50 μg protein/ml) and [^{3}H]oleate (326 nM oleic acid, 6 μCi/ml). Lipids were then extracted, and the radioactivity in cholesteryl ester (CE) and triacylglycerol (TG) was determined. Data are means from 3 experiments in duplicate, and error bars show SE. *$P < 0.05$ vs. corresponding control.

Synthesis of CE was consistently lower in the presence of lycopene (−17 to 33%) under all conditions (Fig. 2*B*), but in this case the changes in the absence of lipoprotein ($P < 0.05$) or in the presence of oxLDL were statistically significant ($P < 0.05$).

Effects of lycopene on scavenger receptor activity in HMDM. Scavenger receptor activity in HMDM was assessed by measuring the internalization of [^{3}H]CE-labeled acLDL or aggLDL. HMDM were incubated with the radiolabeled lipoproteins (100 μg protein/ml) for 24 h in the presence or absence of lycopene (10 μM), and the radioactivity in cellular cholesterol was then determined. There was a decrease of a similar magnitude in the uptake of both acLDL (−28.7%) and aggLDL (−26.5%) when lycopene was present in the incubations (Table 2). Because of the variability in the individual values obtained with aggLDL, however, only the change observed with acLDL was statistically significant ($P < 0.05$).

Effects of lycopene on the expression of mRNA for scavenger receptors in THP-1 macrophages treated with nLDL or modified LDL. THP-1 macrophages were incubated with or without (DMSO) lycopene (10 μM) for 24 h, followed by a further 24 h in the presence or absence of nLDL, acLDL, or aggLDL, and the levels of mRNA for the scavenger receptors SR-B1, CD36, and SR-A were determined by qPCR. The results are expressed as the degree of change compared with incubations without DMSO, lycopene, or lipoproteins, and the results are presented in Fig. 3. DMSO alone had little effect on the abundance of transcripts for CD36 or SR-A but decreased levels of SR-B1 mRNA by ~50%. Lycopene treatment caused a further significant decrease in mRNA for SR-B1 in the presence of nLDL ($P < 0.05$), but not the other LDL types tested (Fig. 3*A*). In contrast, levels of mRNA for CD36 in incubations with nLDL were significantly elevated by lycopene ($P < 0.01$), although again no change was observed when other types of LDL were used. In the case of SR-A, mRNA abundance was unaffected by lycopene in the absence of lipoprotein or in the presence of nLDL but was downregulated in lycopene-treated cells when modified LDL was present, and this difference was significant with aggLDL ($P < 0.05$; Fig. 3C).

Effects of lycopene on cytokine secretion by HMDM treated with nLDL or modified LDL. After incubation of HMDM with or without lycopene for 24 h, the cells were washed and incubated for a further 24 h in the absence or presence of nLDL or aggLDL, and the secretion of proinflammatory (TNF-α and IL-1β) and anti-inflammatory (IL-10) cytokines was measured. Lycopene had no significant effect on the production of IL-1β or TNF-α (Fig. 4, *A* and *B*), but the production of the anti-inflammatory cytokine IL-10 was markedly reduced to a similar extent (−62 to 74%) regardless of whether nLDL or aggLDL was present in the incubation (nLDL, $P < 0.05$; aggLDL, $P < 0.01$; without LDL, $P < 0.05$; Fig. 4*C*).

DISCUSSION

Evidence from epidemiological and other studies suggests that consumption of lycopene in tomatoes and tomato products is associated with decreased risk of CVD (3, 8, 33, 37). Fuhrman et al. (13) have found reduced plasma LDL cholesterol levels in men fed a dietary supplement of tomato lycopene for 3 mo, and this hypocholesterolemic effect, together with the inverse relationship between its serum or tissue levels and intimal wall thickness (3, 33, 37), suggest that this protection may be related to retardation of the development of atherosclerosis. Macrophage foam cell formation in the early stages of atherogenesis is associated with the unregulated uptake of oxidized or modified LDL (1, 46), resulting in the accumulation of large amounts of lipid, particularly CE. In addition,

Table 2. *Effects of lycopene on scavenger receptor activity in HMDM*

Modified LDL	Control, pmol·h^{-1}·mg protein^{-1}	+Lycopene	
		pmol·h^{-1}·mg protein^{-1}	%Control
acLDL	6,150±298	4,444±607*	71.3±6.6
aggLDL	14,925±3,518	9,605±1,300	73.5±9.7

Data shown are means ± SE from 5 experiments in duplicate. HMDM, human monocyte-derived macrophages; LDL, low-density lipoprotein; acLDL and aggLDL, LDL modified by acetylation and aggregation, respectively. HMDM were incubated with [^{3}H]cholesteryl ester (CE)-acLDL or -aggLDL for 24 h with [^{3}H]CE-acLDL (100 μg protein/ml; 156–157,000 disintegrations·min^{-1}·well^{-1}) each in combination with either DMSO (control) or lycopene (10 μM). The lipids were then extracted, and the radioactivity associated with cellular cholesterol and CE was determined. Total labeled cholesterol taken up by HMDM was calculated as the sum of the radioactivity associated with the [^{3}H]cholesterol and [^{3}H]CE bands. *$P < 0.05$ vs. control (Student's paired *t*-test).

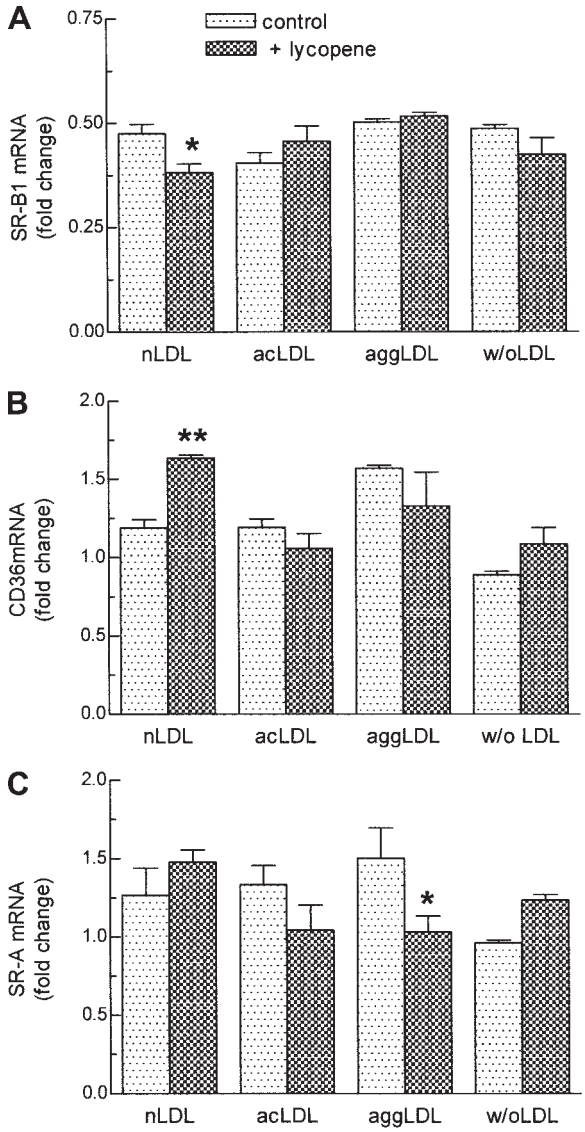

Fig. 3. Effects of lycopene on the expression of mRNA for scavenger receptors in THP-1 macrophages treated with nLDL or modified LDL. THP-1 macrophages were incubated with lycopene (10 μM), DMSO (control), or without additions for 24 h. nLDL, acLDL, aggLDL (100 μg protein/ml), or the vehicle only was then added, and the incubations continued for a further 24 h. After this time, total RNA was extracted from the cells, and the abundance of transcripts for SR-A, SR-B1, and CD36 was determined by RT-PCR. Data are expressed as degree of change compared with incubations without DMSO, lycopene, or lipoproteins and are means from 3 experiments in duplicate. Error bars show SE. $*P < 0.05$ and $**P < 0.01$ vs. corresponding control.

increased synthesis of CE and triacylglycerols, changes in mRNA expression for genes involved (7), and altered inflammatory responses (31, 36) are observed. The antioxidant properties of lycopene (12) suggest that it may influence these processes. In this study, therefore, we have investigated the influence of lycopene on cholesterol accumulation, lipid synthesis, scavenger receptor activity, and mRNA expression and cytokine production in HMDM.

The effects of lycopene on LDL-induced cholesterol accumulation in macrophages have not been reported previously, but other antioxidants such as vitamin E and probucol have given mixed results, with decreased (11, 41, 48), unchanged (5, 45), and increased (43, 45) macrophage cholesterol accumulation being reported. Recent work from our laboratory, however, has shown that FeAOX-6, a synthetic compound that combines the antioxidant structural features of vitamin E and lycopene, decreased the cholesterol content of HMDM in the absence of LDL, although there was little effect in the presence of nLDL, acLDL, or oxLDL (27). In the present study, cholesterol accumulation in HMDM was unaffected by lycopene in the absence of LDL but tended to be decreased when LDL or modified LDL (acLDL or aggLDL) was present (Fig. 1). The differences were significant only when the results from all types of LDL were combined, suggesting that, as with other antioxidants such as FeAOX-6, the inhibitory effects of lyco-

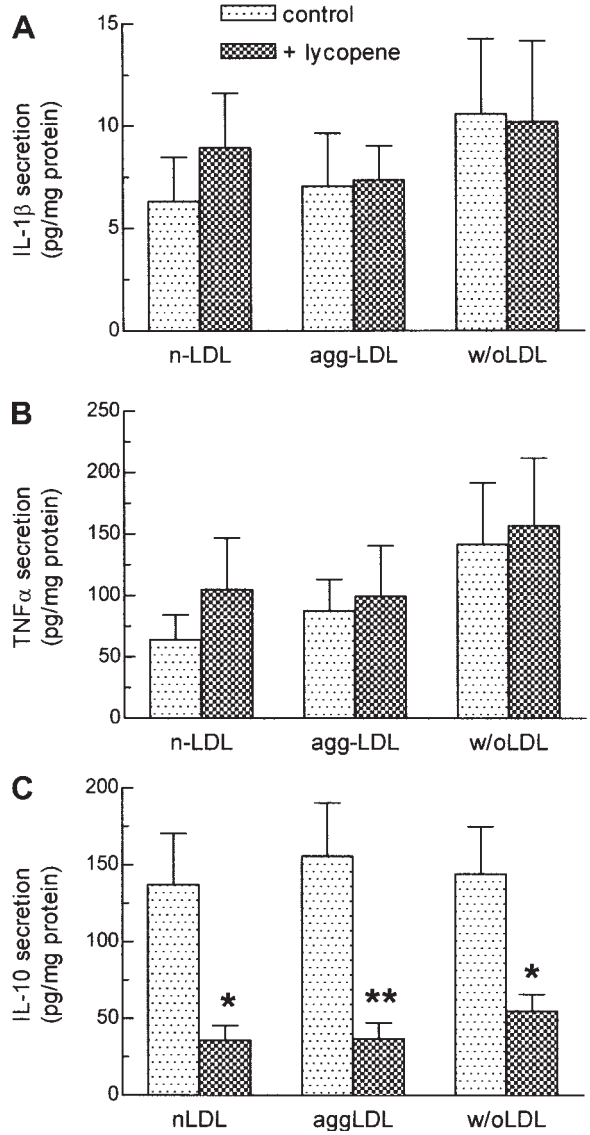

Fig. 4. Effects of lycopene on cytokine secretion by HMDM treated with nLDL or modified LDL. HMDM were pretreated with lycopene (10 μM) or DMSO for 24 h, and the cells were washed and incubated for a further 24 h with lycopene or DMSO (control) in the presence or absence of nLDL or aggLDL (100 μg protein/ml). The medium was then collected, and concentrations of TNF-α, IL-1β, and IL-10 were determined using enzyme-linked immunosorbent assays. Data are means from 3 experiments in duplicate, and error bars show SE. $*P < 0.05$ and $**P < 0.01$ vs. corresponding control.

pene on LDL-induced cholesterol accumulation in macrophages are marginal.

The influx of cholesterol in macrophages during foam cell formation is usually accompanied by an increase in the rate of the conversion of cholesterol to CE for storage in the cytosol (42). In our experiments, the rate of cholesterol esterification in HMDM was decreased by lycopene, irrespective of the presence of lipoproteins, reaching significance in the absence of LDL and with oxLDL (Fig. 2*B*). These results are consistent with previous findings with vitamin E (38, 41) and are broadly similar to those obtained using comparable concentrations of FeAOX-6, where reductions in CE synthesis in HMDM were most marked in the presence of oxLDL compared with nLDL or acLDL (27). α-Tocotrienol, a vitamin E derivative, however, caused greater decreases in all cases (27). In experiments with the murine macrophage cell line J774, Furhman et al. (13) have found that a comparable concentration of lycopene to that used here decreased cholesterol synthesis via inhibition of the rate-limiting enzyme for the pathway, 3-hydroxy-3-methylglutaryl reductase, and this may contribute to the decreased rate of cholesterol esterification. The synthesis of triacylglycerol was also decreased by lycopene in the presence, but not the absence, of lipoprotein, and the effect was greater with modified LDLs (Fig. 2*A*). We conclude, therefore, that lycopene inhibits lipid synthesis in macrophages and that this effect is enhanced in the presence of modified LDL.

Because uptake of LDL via the LDLr is downregulated by the influx of cholesterol in macrophages, the massive accumulation that occurs in foam cell formation is dependent on uptake of modified LDL by the unregulated scavenger receptors (10). Macrophages express a number of scavenger receptors, including SR-A, SR-B1, and CD36 (23), with SR-A and CD36 thought to be the main receptors responsible for the uptake of modified LDLs (21). Vitamin E has been shown to reduce the activity and expression of SR-A and CD36 in macrophages (14, 36, 44), but the effects of lycopene are not known. The results presented here show that scavenger receptor activity, as assessed by the uptake of [^{3}H]CE by HMDM after incubation with [^{3}H]CE-acLDL or aggLDL, was decreased by lycopene. Furthermore, the downregulation of the expression of mRNA for SR-A by lycopene in THP-1 macrophages incubated with modified LDLs (Fig. 3) suggests that this effect may be because of reduced uptake via this receptor, since CD36 mRNA abundance was unaffected under these conditions and increased in the presence of nLDL (Fig. 3). These results are consistent with our previous finding that SR-A, but not CD36, mRNA expression is downregulated in macrophages lipid loaded with chylomicron remnant-like particles (CRLPs) containing lycopene or probucol compared with control CRLPs, whereas CD36 mRNA levels are unchanged (24). In view of this decrease in scavenger receptor activity and mRNA expression, it is perhaps surprising that we found only a marginal decrease in intracellular cholesterol accumulation induced by modified LDL (Fig. 1). However, because the radiolabeling technique used to measure scavenger receptor activity is much more sensitive than the cholesterol mass measurements, changes in the latter parameter are likely to be more difficult to detect.

SR-B1 has a dual role in macrophage lipoprotein metabolism; it is believed to play a part in the uptake of lipid from oxLDL, but its main role is to facilitate the efflux of cholesterol from the cells to high-density lipoprotein (30). The influence of lycopene or other antioxidants on SR-B1 expression in macrophages has not been reported previously. Our experiments show little effect of lycopene on macrophage SR-B1 mRNA levels in the absence of LDL or in the presence of modified LDLs, whereas there was a modest decrease when nLDL was present, suggesting that cholesterol efflux from the cells may be lowered in these conditions.

It has been suggested that inhibition of proinflammatory cytokine secretion by macrophages may be one of the mechanisms mediating the beneficial effects of antioxidants on atherosclerosis development (26, 39). This idea is based primarily on studies with vitamin E, which has been shown to reduce the secretion of inflammatory cytokines, including IL-1β, IL-6, and TNF-α, by monocytes and macrophages (19, 39). The effects of lycopene on cytokine secretion by macrophages, however, have not been studied previously. In our experiments, we found no significant effect of lycopene on the production of TNF-α or IL-1β by HMDM in the absence of LDL or in the presence of either nLDL or modified LDL (Fig. 4, *A* and *B*).The secretion of the anti-inflammatory IL-10, however, was markedly reduced by up to 75% by lycopene in all three conditions (Fig. 4*C*). This effect on IL-10 release is similar to that observed with FeAOX-6 (without added lipoprotein) in our previous study (27) and suggests that lycopene induces a more pro- than anti-inflammatory profile of cytokine secretion by macrophages, regardless of the presence of LDL.

Lycopene is a powerful antioxidant and has the strongest singlet oxygen capacity of a number of common plant carotenoids, including β-carotene (3, 12). Attention has focused on the antioxidant properties of lycopene as a possible explanation for its reported beneficial effects in the prevention of CVD (34, 37, 47), and some studies support this idea (3, 47). However, there is evidence to suggest that other factors may be involved. Plasma lycopene concentrations are similar in smokers and nonsmokers, although smokers are in a higher oxidative state, and higher lycopene concentrations tend to be most protective against myocardial infarction in subjects who have never smoked (3). Previous work has shown that lycopene suppresses cholesterol synthesis in macrophages but increases LDLr activity (13). In the present study, lycopene treatment resulted in decreased lipid synthesis, scavenger receptor activity, and SR-A mRNA expression and caused a small reduction in cholesterol accumulation in macrophages exposed to modified LDLs, effects that have been reported to occur with other antioxidants such as vitamin E, FeAOX-6, and/or probucol (11, 14, 27, 38, 44, 48). On the other hand, in contrast to vitamin E, lycopene did not decrease CD36 mRNA expression and also tended to cause proinflammatory rather than anti-inflammatory changes in cytokine secretion (11, 14, 19, 39, 44). It seems likely, therefore, that, while some of the effects of lycopene on foam cell formation are related to its antioxidant activity, others are caused by different mechanisms.

In conclusion, the results of this study indicate that lycopene may reduce macrophage foam cell formation in response to modified LDL by decreasing lipid synthesis in the cells and downregulating the activity and expression of SR-A. These potentially beneficial effects, however, are accompanied by a marked decrease in the secretion of the anti-inflammatory cytokine IL-10, resulting in an increase in the proinflammatory profile of macrophage cytokine release.

These findings provide new information about the role of lycopene in modulating the cellular events leading to atherosclerosis development.

ACKNOWLEDGMENTS

We thank Graziano Santucci for help with the analyses.

REFERENCES

1. **Albertini R, Moratti R, DeLuca G.** Oxidation of low-density lipoprotein in atherosclerosis from basic biochemistry to clinical studies. *Curr Mol Med* 2: 579–592, 2002.
2. **Amundson DM, Zhou M.** Fluorometric method for the enzymatic determination of cholesterol. *J Biochem Biophys Methods* 38: 43–52, 1999.
3. **Arap L, Steck S.** Lycopene and cardiovascular disease. *Am J Clin Nutr Suppl* 71: 1691S–1695S, 2000.
4. **Asmis R, Jelk J.** Large variation in human foam cell formation in individuals: a fully autologous in vitro assay based on the quantitiative analysis of cellular neutral lipids. *Atherosclerosis* 148: 243–253, 2000.
5. **Asmis R, Jelk J.** Vitamin E supplementation of human macrophages prevents neither foam cell formation nor increased susceptibility of foam cells to lysis by oxidized LDL. *Arterioscler Thromb Vasc Biol* 20: 2078–2086, 2000.
6. **Basu SK, Goldstein JL, Brown MS.** Receptor-mediated endocytosis of low density lipoprotein in cultured cell. *Meth Enzymol* 46: 241–261, 1986.
7. **Batt KV, Patel L, Botham KM, Suckling KE.** Chylomicron remnants and low density lipoprotein have differential effects on the expression of mRNA for genes involved in human macrophage formation. *J Mol Med* 82: 449–458, 2004.
8. **Blum A, Monir M, Wirsansky I, Ben-Arzi S.** The beneficial effects of tomatoes. *Eur J Int Med* 16: 402–404, 2005.
9. **Bradford M.** A sensitive method for quantitation of microgram quantities of protein utilizing the principle of dye-binding. *Anal Biochem* 72: 248–252, 1976.
10. **Brown MS, Goldstein JL.** Lipoprotein metabolism in the macrophages: implications for cholesterol deposition in atherosclerosis. *Ann Rev Biochem* 52: 223–261, 1983.
11. **Devaraj S, Hugou I, Jialal I.** Alpha tocopherol decreases CD36 expression in human monocyte-derived macrophages. *J Lipid Res* 42: 521–527, 2001.
12. **Di Mascio P, Kaiser S, Sies H.** Lycopene as the most efficent biological carotenoid single oxygen quencher. *Arch Bochem Biophys* 274: 532–538, 1989.
13. **Furhman B, Elis A, Aviram M.** Hypocholesterolemic effect of lycopene and beta carotene is related to suppression of cholesterol synthesis and augmentation of LDL receptor activity in macrophages. *Biochem Biophys Res Commun* 233: 658–662, 1997.
14. **Furhman B, Volkova N, Aviram M.** Oxidative stress increases the expression of the CD36 scavenger receptor and the cellular uptake of oxidized low-density lipoprotein in macrophages from atherosclerotic mice: protective role of antioxidants and of paraoxonase. *Atherosclerosis* 161: 307–316, 2002.
15. **Gebicki J, Jurgens G, Esterbauer H.** Oxidation *of* low density lipoprotein in vitro, In: *Oxidative Stress,* edited by Sies H. New York: Academic, 1991, p. 371–397.
16. **Guyton JR, Klemp KF, Mims MP.** Altered ultrastructural morphology of self-aggregated low density lipoproteins: coalescence of lipid domains forming droplets and vesicles. *J Lipid Res* 32: 953–962, 1991.
17. **Heber D, Lu QY.** Overview of mechanisms of action of lycopene. *Exp Biol Med (Maywood)* 227: 920–923, 2002.
18. **Huang W, Ishii I, Zhang WY, Sonobe M, Kruth HS.** PMA activation of macrophages alters macrophage metabolism of aggregated LDL. *J Lipid Res* 43: 1275–1282, 2002.
19. **Jung WJ, Sung MK.** Effects of major dietary antioxidants on inflammatory markers of RAW 264.7 macrophages. *Biofactors* 21: 113–117, 2004.
20. **Kok FJ, Kromhout D.** Atherosclerosis- epidemiological studies on the health effects of a Mediterranean diet. *Eur J Nutr* 43, *Suppl* 1: 2–5, 2004.
21. **Kunjathoor VV, Febbraio M, Podrez EA, Moore KJ, Abdersson L, Koehn L, Rhee JS, Siverstein R, Hoff HF, Freeman MW.** Scavenger receptors class A-I/II and CD36 are the principal receptors responsible for the uptake of miodificed low density lipoprotein leading to lipid loading in macrophages. *J Biol Chem* 277: 49982–49988, 2002.
22. **Libby P, Geng YJ, Aikawa M, Schoenbeck U, Mach F, Clinton SK, Sukhova GK, Lee RT.** Macrophages and atherosclerotic plaque stability. *Curr Opin Lipidol* 7: 330–335, 1996.
23. **Matsuura E, Kobayashi K, Tabuchi M, Lopez LR.** Oxidative modification of low-density lipoprotein and immune regulation of atherosclerosis. *Prog Lipid Res* 45: 466–486, 2006.
24. **Moore EH, Napolitano M, Avella M, Bejta F, Suckling KE, Bravo E, Botham KM.** Protection of chylomicron remnants from oxidation by incorporation of probucol into the particles enhances their uptake by human macrophages and increases lipid accumulation in the cells. *Eur J Biochem* 271: 2417–2427, 2004.
25. **Moore EH, Napolitano M, Prosperi A, Avella M, Suckling KE, Bravo E, Botham KM.** Incorporation of lycopene into chylomicron remnant-like particles enhances their induction of lipid accumulation in macrophages. *Biochem Biophys Res Commun* 312: 1216–1219, 2003.
26. **Munteanu J, Zingg M, Azzi A.** Anti-atherosclerotic effects of vitamin E -myth or reality? *J Cell Mol Med* 8: 59–76, 2004.
27. **Napolitano M, Avanzi L, Manfredi S, Bravo E.** Effects of a new combinative antioxidant FeAOX-6 and alpha toctrienol on macrophage atherogenesis-related functions. *Vascul Pharmacol* 46: 394–405, 2007.
28. **Napolitano M, Bravo E.** Activation of protein Kinase C by phorbol esters in human macrophages reduces the metabolism of modified LDL by down-regulation of scavenger receptor activity. *Int J Biochem Cell Biol* 35: 1127–1143, 2002.
29. **Napolitano M, Bravo E.** Lipid metabolism and TNF-alpha secretion in response to dietary sterols in human monocyte derived macrophages. *Eur J Clin Invest* 35: 482–490, 2005.
30. **Ohashi R, Mu H, Wang X, Yao Q, Chen C.** Reverse cholesterol transport and cholesterol efflux in atherosclerosis. *Q J Med* 98: 845–856, 2005.
31. **Persson J, Nilsson J, Lindholm MW.** Cytokine response to lipoprotein loading in human monocyte-derived macrophages (Abstract). *Lipids Health Dis* 5: 17, 2006.
32. **Pfaffl MW.** A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2: 2002–2007, 2001.
33. **Rao AV, Ray MR, Rao LG.** Lycopene. *Adv Food Nutr Res* 51: 99–164, 2006.
34. **Rao AV, Agarwal S.** Bioavailability and in vivo antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. *Nutr Cancer* 31: 199–203, 1998.
35. **Riso P, Visioli F, Grande S, Guarnieri S, Gardana C, Simonetti P, Porrini M.** Effect of a tomato-based drink on markers of inflammation, immunomodulation and oxidative stress. *J Agric Food Chem* 54: 2563–2566, 2006.
36. **Robbeysin F, Salvayre R, Negre-Salvayre A.** Dual role of oxidized LDL on the NK-kappaB signalling pathway. *Free Rad Res* 38: 541–551, 2004.
37. **Sesso HD.** Carotenoids and cardiovascular disease:what research gaps remain? *Curr Opin Lipidol* 17: 11–16, 2006.
38. **Shige H, Ishikawa T, Suzukawa M, Nishiwaki M, Yamashita T, Nakajima K, Ito T, Higashi K, Ayaori M, Yonemura A, Nestel P, Nakamura H.** Vitamin E reduces cholesterol esterification and uptake of acetylated low density lipoprotein in macrophages. *Lipids* 33: 1169–1175, 1998.
39. **Singh U, Deveraj S, Jialal I.** Vitamin E, oxidative stress and inflammation. *Annu Rev Nutr* 25: 151–174, 2005.
40. **Steinberg D.** Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. *Nat Med* 8: 1211–1217, 2002.
41. **Suzukawa M, Abbey M, Clifton P, Nestel PJ.** Effects of supplementing with vitamin E on the uptake of low density lipoprotein and the stimulation of cholesteryl ester formation in macrophages. *Atherosclerosis* 110: 77–86, 1994.
42. **Suzuki H, Kurihara Y, Takeya M, Kamada N, Kataoka M, Jishage K, Sakaguchi H, Kruijt JK, Higashi T, Suzuki T, van Berkel TJ, Horiuchi S, Takahashi K, Yazaki Y, Kodama T.** The multiple roles of macrophage scavenger receptors (MSR) in vivo: resistance to atherosclerosis and susceptibility to infection in MSR knockout mice. *J Atheroscler Thromb* 4: 1–11, 1997.
43. **Takemura T, Sakai M, Matsuda H, Matsumura T, Biwa T, Anami Y, Nishikawa T, Sasahara T, Shichiri M.** Effects of probucol on cholesterol metabolism in mouse peritoneal macrophages: inhibition of HDL mediated cholesterol efflux. *Atherosclerosis* 152: 347–357, 2000.
44. **Teupser J, Thiery D, Seidel D.** α-Tocopherol down-regulates scavenger receptor activity in macrophages. *Atherosclerosis* 144: 109–115, 1999.

45. **Trach CC, Wulfroth PM, Severs NJ, Robenek H.** Influence of native and modified lipoproteins on migration of mouse peritoneal macrophages and the effect of the antioxidants vitamin E and probucol. *Eur J Cell Biol* 71: 199–205, 1996.
46. **Vainio S, Ikonen E.** Macrophage cholesterol transport: a critical player in foam cell formation. *Ann Med* 35: 146–155, 2003.
47. **Voutilainen S, Nurmi T, Mursu J, Rissanen TH.** Carotenoids and cardiovascular health. *Am J Clin Nutr* 83: 1265–1271, 2006.
48. **Yamamoto A, Hara H, Takaichi S, Wakasugi S, Tomikawa M.** Effect of probucol on macrophages, leading to regression of xanthomas and atheromatous vascular lesions. *Am J Cardiol* 62: 31B–36B, 1988.