A Toll-like receptor 9-mediated pathway stimulates perilipin 3 (TIP47) expression and induces lipid accumulation in macrophages

Jian-Qiu Gu,1* Di-Fei Wang,1* Xiao-Guang Yan,1 Wei-Li Zhong,1 Jin Zhang,1 Bin Fan,2 and Shoichiro Ikuyama2
1Department of Endocrinology and Metabolism, The First Affiliated Hospital, China Medical University, Shenyang, China; and 2Division of Clinical Immunology, Department of Immunobiology and Neuroscience, Medical Institute of Bioregulation, Kyushu University, Beppu, Japan

Submitted 15 March 2010; accepted in final form 12 July 2010

Gu JQ, Wang D, Yan X, Zhong W, Zhang J, Fan B, Ikuyama S. A Toll-like receptor 9-mediated pathway stimulates perilipin 3 (TIP47) expression and induces lipid accumulation in macrophages. Am J Physiol Endocrinol Metab 299: E593–E600, 2010. First published July 13, 2010; doi:10.1152/ajpendo.00159.2010.—Excessive accumulation of lipids in macrophages results in formation of foam cells and is a hallmark of atherosclerotic plaques. The PAT family of proteins has been implicated in this process, but details of their involvement in foam cell formation have not been fully elucidated. One dominant member of the PAT proteins, perilipin 3 (TIP47), is likely to be involved in such a regulatory mechanism. In this study, we demonstrated that the Toll-like receptor 9 (TLR9)-mediated pathway stimulates perilipin 3 expression and accumulation of lipids, especially triglycerides, in macrophages. Oligodeoxynucleotide (ODN) 1826, a ligand of TLR9, significantly enhanced perilipin 3 expression in RAW264.7 cells, and chloroquine, a TLR9 inhibitor, almost completely inhibited ODN1826-induced perilipin 3 expression. The inhibitors of c-jun NH2-terminal kinase and PI 3-kinase suppressed the level of perilipin 3 mRNA induced by ODN1826. ODN1826 induced the expression of IL-1α and IFNβ, both of which increased perilipin 3 expression. Antibodies against these cytokines suppressed the ODN1826-induced perilipin 3 mRNA levels. These results suggest that the expression of perilipin 3 in macrophages is in part regulated through the TLR9-mediated mechanism. Furthermore, ODN1826 increased intracellular lipid accumulation in the presence of oxLDL, which was reduced by perilipin 3 siRNA. Perilipin 3 expression was not stimulated by oxLDL. Depletion of perilipin 3 by siRNA specifically reduced triglyceride content in the cells but not cholesterol content, indicating that perilipin 3 is involved mainly in triglyceride accumulation. In conclusion, the TLR9-mediated pathway facilitates foam cell formation in part through increased expression of perilipin 3.

ODN1826; PAT protein family; atherosclerosis; tail-interacting protein of 47 kDa

THE FORMATION OF FOAM CELLS is a hallmark of early atherosclerosis. The uptake of lipids by macrophages results in formation of intracellular lipid droplets (LDs), which is a critical process not only to atherosclerosis but also to obesity, diabetes, and hepatic steatosis (21, 27). It has been found that a variety of proteins exist on the surface of intracellular LDs that participate in lipid synthesis, storage, utilization, and degradation (27). Among them, the most abundant LD-associated proteins are the members of PAT protein family, named after their constituents perilipin, adipose differentiation-related protein (ADRP), and tail-interacting protein of 47 kDa (TIP47); they have recently been redefined as perilipin 1, perilipin 2, and perilipin 3 (18), respectively. Among these proteins, perilipin 2 (ADRP) and perilipin 3 (TIP47) are ubiquitously expressed in a variety of cells (2, 37, 38). Perilipin 2 is expressed predominantly in macrophages and foam cells at the site of atherosclerotic lesion (19, 20, 35). Importantly, perilipin 2 gene abrogation significantly prevented atherosclerosis formation in apolipoprotein E-knockout mice (29). Perilipin 3 was described originally as a cytosolic protein that binds to the cytoplasmic domains of the cation-dependent and cation-independent mannose 6-phosphate receptors (8). Perilipin 3 was also shown to be located on the LD surface of HeLa cells and other cell types (30, 33, 37). Based on the high structural homology, potential function, and tissue distribution of perilipin 2 and perilipin 3, it has been suggested that perilipin 3 may play a similarly important pathophysiological role in atherosclerogenesis. Indeed, a recent study has demonstrated that perilipin 3 acts as a carrier protein of free fatty acids and is involved in foam cell formation (4). However, the regulatory mechanism and effect of perilipin 3 on foam cell formation has not been fully investigated.

Macrophages are stimulated by pathogen-associated molecules through the Toll-like receptors (TLRs) (31). Human and mouse TLRs consist of a large family with at least 13 members (3) that are known to recognize different molecules of microbial origin as well as a variety of endogenous ligands released during the process of disease (28, 39). Previous reports suggested that myeloid differentiation factor 88 (MyD88)-dependent TLR signaling plays an important role in the development of atherosclerosis (1). Other animal studies have also shown that TLR2 and TLR4 are involved in the development of atherosclerotic plaques (24, 26). Although a recent study has demonstrated that TLR9 signaling promotes transformation of macrophages to foam cells (23), little is known about the mechanism by which TLR9-mediated signaling pathway leads to foam cell formation and atherosclerosis. Therefore, in the present study, we aimed to determine whether TLR9 signaling can regulate the expression of perilipin 3 in macrophages and whether this process results in foam cell formation. We demonstrate here that oligodeoxynucleotide (ODN) 1826, a TLR9 ligand, enhances the perilipin 3 expression and in turn promotes accumulation of lipids, specifically triglyceride, in macrophages.

MATERIALS AND METHODS

Reagents. Mouse recombinant IL-1α, IL-1β, IL-5, IL-6, TNFα, IFNα, IFNβ, thioglycollate, chloroquine (CQ), and LY-20094 were purchased from Sigma-Aldrich (St. Louis, MO), U-0126, SB-203580, and SP-600125 were purchased from CalBiochem (San Diego, CA).
Antibodies against perilipin 3 and GAPDH were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against mouse IL-1α, INFγ, normal rat IgG2b, and rabbit IgG were purchased from R & R Systems (Minneapolis, MN). ODN1826 was purchased from Invivogen (San Diego, CA). Oxidized LDL (oxLDL) was purchased from Shanghai Maisha Biotech (Shanghai, China). siRNA was purchased from Qiagen (Hilden, Germany) (HP-guaranteed siRNA). A sequence-specific siRNA for perilipin 3 (sense: 5′-AA-CAGCACAGAGAUGAGGAG-3′) was selected by its potency.

**Cell culture.** The mouse RAW264.7 macrophage cell line was obtained from American Type Culture Collection (Manassas, VA). The cells were routinely cultured in 10-cm tissue culture dishes in α-MEM (Sigma-Aldrich) supplemented with 10% charcoal-treated fetal calf serum (FCS), 1% nonessential amino acids, and appropriate antibiotics. The FCS we used was proven to not be influenced by charcoal treatment in terms of the concentrations of fatty acids, triglyceride, and total cholesterol (13, 36). The cells were cultivated in the same medium, except for the lipid-free FCS (designated as lipid-free medium; Sigma-Aldrich) in the experiments testing oxLDL effects.

**Western blot analysis.** The cells were harvested using RIPA lysis buffer containing 50 mM Tris·HCl (pH 7.4), 150 mM NaCl, 1% sodium deoxycholate, 1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, and 1 mM EDTA. The proteins were heated at 95°C for 5 min and then applied to 10% SDS-PAGE and electroblotted onto a polyvinylidene difluoride membrane (MSI, Westborough, MA) for 1 h at 100 V with a Western blotting apparatus (Bio-Rad, Hercules, CA) in Tris-glycine transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, and 0.1% SDS). The membranes were blocked for 1 h at room temperature with 5% nonfat milk in PBS containing 0.1% Tween 20 (PBS-Tween 20). Next, the membranes were incubated with rabbit perilipin 3 antibodies (0.5 μg/ml in PBS-Tween 20) for 1 h. After being washed four times (5 min each) with PBS-Tween 20, the membranes were incubated with an appropriate secondary IgG-horseradish peroxidase conjugate (Santa Cruz Biotechnology) diluted in PBS-Tween 20 (0.08 μg/ml) for 1 h and then washed as described above. The immunoreactivity was detected by the enhanced chemiluminescence technique. Three independent experiments were carried out, and a representative example has been shown.

**Real-time PCR.** Total RNA was extracted using TriReagent (Sigma-Aldrich) according to the manufacturer’s instructions. Single-strand cDNA was synthesized with ReverTra Ace (Toyobo, Osaka, Japan), using 0.5 μg of the total RNA. Real-time PCR was performed with the SYBR Green method using the ABI prism 7500 Sequence Detection System (Applied Biosystems, Foster City, CA) in 25-μl reactions [12.5 μl of 2 × IQ SYBR Green Supermix (Bio-Rad), 320 nM for each primer, 5 μl of 1:20 diluted cDNA]. The primer sequences were perilipin 3 (sense: CTGTCCTACACAGCTCTGCT; antisense: CGATGCTTCTCTTCCTACCC), IL-1α (sense: GATGCCTTCAGGTGCGAC; antisense: GAATCTTCCGTGGTCTTGAC), INFγ (sense: TAAGCAGTCCGAGACGCTTAC; antisense: GAGACATCTCCAGCTGAC-TC), perilipin 2 (sense: CTGTCCTACACAGCTCTGCT; antisense: CGATGCTTCTCTTCCTACCC), and GAPDH [sense: ACCACGTCATGCATCAC; antisense: TCCACACCCTGGT-GCCTTA]. PCR efficiencies for all reactions were >0.90. Quantitative PCR results have been expressed as relative induction fold in correspondence to the housekeeping gene GAPDH.

**Oil red O staining.** Cells were plated in a four-well chamber slide (Nalgene Nunc International, Naperville, IL) at 1 × 10^5 cells/chamber. After treatment, the cells were washed with PBS, fixed in 10% formaldehyde, and stained with the neutral lipid dye Oil red O (0.3% in 60% isopropanol), followed by extensive washes (10). The intracellular lipid droplets were then detected under a light microscope (LABOPHOT2A; Nikon).

**Transient transfection.** siRNAs were introduced into RAW264.7 cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. The cells were grown in four-well chamber slides for 24 h to 50% confluence before transfection. The medium was changed to serum-depleted α-MEM, and the transfection mixture (1 μl of Lipofectamine 2000 and 40 pmol of siRNA in 50-μl medium) was added to the cells, followed by incubation for 4 h. The cells were then washed with serum-depleted α-MEM and cultured in normal α-MEM. The cells were allowed to knock down perilipin 3 for 72 h before analysis by Western blot or Oil red O staining.

**Lipid analysis.** Cells were incubated with or without oxLDL in lipid-free medium for 24 h before harvest. Cells were washed twice by PBS and then scraped in 400 μl of doubly distilled H2O. After cells were sonicated, the protein content of the lysate was measured using the Bradford protein assay (Bio-Rad). Triglyceride content was measured using an enzymatic method (Sigma-Aldrich), as described previously (15). Total cholesterol and free cholesterol in cells were determined using the fluorometric method of Gamble et al. (12) for aqueous cell homogenates. Cholesterol ester was measured as the difference between total and free cholesterol. Lipid contents were standardized by protein concentrations.

**Isolation of mouse peritoneal macrophages.** Female C57BL/6N mice at the age of 8–10 wk were purchased by the experimental animal centre of China Medical University. All animal experiments were conducted in accordance with the China Medical University...
ODN1826-stimulated expression of perilipin 3. The expression of perilipin 3 mRNA (Fig. 2A) and protein (Fig. 2B) was blocked significantly when the cells were pretreated with CQ (5 µg/ml). These findings suggest that the TLR9-mediated pathway is involved in the regulation of perilipin 3 expression in RAW264.7 macrophages.

**RESULTS**

TLR9-mediated pathway is involved in the enhancement of perilipin 3 expression. Since TLR9 activation is known to induce foam cell transformation from macrophages (23), we first tested whether ODN1826, a TLR9 ligand, could stimulate the expression of perilipin 3 in RAW264.7 cells. ODN1826 enhanced the expression of the perilipin 3 mRNA in a time- and dose-dependent manner (Fig. 1, A and B). Western blot analysis confirmed that ODN1826 increased the perilipin 3 protein level as well (Fig. 1C). Since TLR ligands function through TLR-dependent pathways (16), we confirmed whether CQ, a specific inhibitor of TLR9, could suppress the ODN1826-stimulated expression of perilipin 3. The expression of perilipin 3 protein level as well (Fig. 1B), were assessed by real-time PCR and Western blot, respectively. PLIN3 protein levels were quantified by NIH Image (Fig. 2, bottom). The PLIN3 mRNA or protein level of the control (without both agents) is arbitrarily designated as 1. The data represent the mean ± SE of 3 independent experiments. *P < 0.05 vs. no treatment; §P < 0.05. ns, Not significant.

**A**

![Graph showing PLIN3 mRNA expression](image1)

**B**

![Graph showing PLIN3 protein level](image2)

**C**

![Graph showing PLIN3 protein level](image3)

To determine whether ODN1826-induced expression of perilipin 3 was mediated by an autocrine mechanism of IL-1α and IFNβ, we examined the effect of ODN1826 on the expression of IL-1α and IFNβ in RAW264.7 cells. ODN1826 significantly induced IL-1α and IFNβ mRNA expression as quickly as 3 h after incubation (Fig. 5, A and B). The cytokine response was more rapid compared with that of perilipin 3 mRNA; the former reached a peak 6 h after stimulation (Fig. 5A vs. Fig. 5, A and B). These results suggest that IL-1α and IFNβ were able to mediate the perilipin 3 expression induced by ODN1826. To
confirm this finding further, we tested the effect of antibodies against IL-1α/H9251 and IFN-β/H9252 on ODN1826-induced perilipin 3 expression. In the presence of either IL-1α or IFN-β antibody, ODN1826 increased the perilipin 3 mRNA level to only 79 and 82%, respectively, of that of the control IgG. The perilipin 3 mRNA level was further reduced to 57% of that of the control when both antibodies were added simultaneously (Fig. 5C). These results indicate that the induction of perilipin 3 expression by ODN1826 is mediated in part by the autocrine mechanism of IL-1α/H9251 and IFN-β/H9252.

TLR9-mediated perilipin 3 expression coincides with lipid accumulation. oxLDL is a potent stimulator of foam cell formation of macrophages (25). To elucidate a potential role of TLR9-mediated perilipin 3 expression in foam cell formation, we tested the effect of ODN1826 and oxLDL in RAW264.7 cells in the lipid-free medium. ODN1826, but not oxLDL, stimulated perilipin 3 mRNA expression ~3.5-fold of the control (Fig. 6A), whereas combination of ODN1826 and oxLDL did not show any additive effect, suggesting again that oxLDL is not involved in perilipin 3 expression. This finding was further confirmed by the perilipin 3 protein levels. ODN1826 increased the protein level to 2.5-fold of the control, but there was no additive effect of the combination (Fig. 6B). Although ODN1826 stimulated perilipin 2 expression (Fig. 8B), it did not induce lipid accumulation in the absence of lipids in the medium, as evidenced by Oil red O staining (Fig. 7A). On the other hand, oxLDL stimulated lipid accumulation in the cells, and combined treatment of ODN1826 and oxLDL further facilitated lipid accumulation (Fig. 7A). To confirm the finding shown above, we measured triglyceride and cholesterol content in the cells (Fig. 7, B and C). ODN1826 alone did not increase

![Fig. 4. Inflammatory cytokines stimulate the PLIN3 expression in RAW264.7 cells. A: RAW264.7 cells were incubated for 24 h with 100 ng/ml IL-1α, IL-1β, IL-5, IL-6, and TNFα and 1.000 U/ml IFNα and IFNβ. The expression of PLIN3 mRNA was assessed by real-time PCR. B and C: RAW264.7 cells were incubated with 1–100 ng/ml IL-1α or 10–1,000 U/ml IFNβ for 24 h. The level of PLIN3 mRNA was determined by real-time PCR. The PLIN3 mRNA level without cytokine stimulation is arbitrarily designated as 1. The results are shown as the mean ± SD of 3 independent experiments (*P < 0.05 vs. 0 ng/ml or 0 U/ml).](chart)

![Fig. 5. IL-1α and IFNβ are involved in ODN1826-induced PLIN3 mRNA expression. A and B: RAW264.7 cells were incubated with 2.5 µg/ml ODN1826 for 0–12 h. The level of mRNA for IL-1α (A) and IFNβ (B) was determined by real-time PCR. The mRNA level at 0 h is arbitrarily designated as 1. The results are shown as the mean ± SD of 3 independent experiments (*P < 0.05 vs. 0 h). C: specific antibodies (Ab) against cytokines suppress the ODN1826-induced PLIN3 mRNA expression. RAW264.7 cells were pre-treated with control immunoglobulin (Ig) or specific antibodies against mouse IL-1α (25 µg/ml) and IFNβ (2 × 10⁵ U/ml) for 1 h and then stimulated with 2.5 µg/ml ODN1826 for 24 h. Concentration of each Ab was the minimal dose that exhibited the maximal effect in preliminary experiments (data not shown). The expression of PLIN3 mRNA was assessed by real-time PCR. Control Ig did not affect the ODN1826 effect. The PLIN3 mRNA level stimulated by ODN1826 in the presence of control Ig is arbitrarily designated as 1. *P < 0.05 vs. ODN, control Ig.](chart)
either triglyceride or cholesterol content compared with the control. On the other hand, oxLDL increased cellular content of cholesterol, but not triglyceride significantly. Interestingly, combination of ODN1826 and oxLDL markedly increased triglyceride content approximately threefold of the control (Fig. 7B). The combination of ODN1826 and oxLDL also increased cholesterol content, especially cholesterol ester level (Fig. 7C). These results apparently supported the finding of Oil red O staining (Fig. 7A). Triglyceride content in the perilipin 3 knocked down cells was decreased to ~50% of that in the control cells (Fig. 8D), whereas cholesterol content was not

Perilipin 3 is involved in the accumulation of triglyceride but not cholesterol. To confirm the above presumption, we performed a series of perilipin 3-knockdown experiments. As shown in previous studies (13, 19, 20, 36), another PAT family protein, perilipin 2, is also involved in foam cell formation of macrophages, and there might be functional interaction between perilipins 2 and 3. To test the possibility first that perilipin 2 might compensate perilipin 3, perilipin 2 mRNA stimulated by ODN1826 was assessed under the condition of siRNA-mediated perilipin 3 knocking down. Transfection of specific siRNA resulted in a 60% decrease in the perilipin 3 protein level compared with the control siRNA (Fig. 8A). In both the presence and absence of perilipin 3 siRNA, the perilipin 2 mRNA expression stimulated by ODN1826 was comparable, approximately threefold of the control (Fig. 8B). This result indicates that perilipin 2 does not show compensatory reduction even in a perilipin 3-depleted condition, although the expression of both perilipins is stimulated by the TLR9-mediated pathway.

When perilipin 3 was knocked down by the specific siRNA, lipid accumulation induced by combination of ODN1826 and oxLDL appeared to be reduced, as visualized by Oil red O staining (Fig. 8C). Triglyceride content in the perilipin 3 knocked down cells was decreased to ~50% of that in the control cells (Fig. 8D), whereas cholesterol content was not
**TLR9 ACTIVATION INDUCES LIPID ACCUMULATION VIA PERILIPIN 3**

**Discussion**

Several lines of evidence have suggested a link between atherosclerosis and the increased expression of TLRs (9, 11, 34). Although excessive intracellular lipid accumulation in macrophages is a key characteristic of the atherosclerotic lesion, and foam cell formation is closely related to LD proteins such as PAT family proteins (19, 20, 29, 35), studies on the TLR-mediated PAT family protein regulation are still very limited. We demonstrated previously the TLR4-mediated perilipin 2 expression and its molecular mechanism in macrophages (13). In the present study, we demonstrated, for the first time, that stimulation of TLR9 induced the expression of perilipin 3, another ubiquitous class of PAT family proteins. We found that rapid production of proinflammatory cytokines such as IL-1α and IFNγ mediated in part the TLR9 signal-induced perilipin 3 expression. In addition, among various intracellular signals evoked by TLR9 stimulation, we identified that JNK and PI3K were dominantly related to this effect.

TLR4 and TLR2 activity are closely related to foam cell formation (5, 7, 16). It has been reported that TLR9 signals stimulate the expression of lectin-like oxLDL receptor-1 (Lox-1) and NADPH oxidase 1 (Nox1) conjointly, which leads to foam cell formation (23). During the preparation of this article, Sorrentino et al. (32) reported that combination of TLR9 ligand and oxLDL highly stimulated foam cell formation in RAW264.7 cells, as we found in the present study. However, neither of the above studies assessed the phenomenon from the LD protein point of view. Important findings in our study are that 1) a TLR9 ligand (ODN1826), but not oxLDL, stimulated perilipin 3 expression and 2) lipid accumulation induced by combination of ODN1826 and oxLDL was reduced by disruption of perilipin 3. More importantly, the present study showed that perilipin 3 is involved mainly in accumulation of triglyceride, possibly derived from oxLDL.

Specific reduction in perilipin 3 by siRNA coincided with the reduction in triglyceride content but not cholesterol content. Perilipin 3 siRNA reduced ODN1826-induced (and oxLDL-induced) triglyceride accumulation to ∼50% of the control. This result appeared to be more dramatic than that shown in the recent report (4). This is probably because the cells were under the ODN1826 stimulation in our study. Perilipin 3 is implicated in transporting free fatty acids to LDs (4), which may in turn promote esterification of cholesterol and accumulate in LDs. Since perilipin 2, which also transports free fatty acids (15), did not exhibit compensatory increase under the perilipin 3 depletion, the triglyceride content in this condition may reflect the net ability of perilipin 2 to accumulate triglycerides induced by TLR9 stimulation. However, it remains undiscovered whether both perilipins could potentiate each other with regard to increases in fatty acid transport when the two proteins coexist. On the other hand, perilipin 2 is known to facilitate cholesterol uptake (20). Therefore, we assume that perilipin 2 could contribute to cholesterol uptake to some extent in the present conditions, possibly in conjunction with other cholesterol uptake mechanisms (23). Although TLR9 activation inhibits cholesterol efflux, thereby affecting cholesterol metabolism, it was shown that TLR9 activation did not suppress cholesterol efflux systems such as ABCA1 (6).

Intracellular signaling pathway evoked by TLR9 activation is the MyD88-dependent pathway (17), through which TLR9 activates complex downstream molecules. Since ERK, p38 MAPK, JNK, and PI3K are representative signaling molecules downstream of TLR9 (14, 17, 22, 28a), we tested which signaling molecules were involved in the perilipin 3 expression. The inhibitors of JNK and PI3K, but not ERK and p38 MAPK, blocked the expression of perilipin 3 mRNA induced...
by ODN1826, suggesting that JNK- and PI3K-mediated pathways regulate the expression. On the other hand, a recent report showed that the TLR9-MyD88-ERK1/2 pathway upregulated early growth response-1, a master transcription factor in atherosclerosis development (17), which in turn promoted foam cell formation (17). It was also shown that TLR9-stimulated expression of Lox-1 and Nox1 was inhibited by p38 MAPK inhibitor (23). These findings, together with our present results, indicate that mechanisms regulating the perilipin 3 expression are distinct from others, although all of these proteins are regulated by TLR9 signals and implicated in foam cell formation.

We reported that LPS/TLR4-induced perilipin 2 expression is mediated in part by an autocrine mechanism of proinflammatory cytokines such as IL-1α, IL-6, and IFNβ in RAW264.7 cells. Therefore, we presumed that perilipin 3 could also be regulated by the similar mechanism and proved that it was the case. Thus, ODN1826 induced the production of IL-1α and IFNβ, and both cytokines significantly enhanced the expression of perilipin 3. The induction of these cytokines by ODN1826 preceded the increase in the perilipin 3 mRNA, and specific antibodies against IL-1α and IFNβ significantly suppressed the ODN1826-induced perilipin 3 expression. However, perilipin 3 was still stimulated even in the presence of sufficient amounts of both antibodies, suggesting that distinct mechanisms are also involved in the regulation. We presume that TLR9-mediated signals directly act on the perilipin 3 gene, as we demonstrated previously in the TLR4-mediated regulation of perilipin 2 gene (13). Another possibility is that other cytokines, which we did not test in this study, might also be involved. Interestingly, we found a difference in the regulation of expression by proinflammatory cytokines for perilipins 2 and 3; IL-6 did not increase the expression of perilipin 3, whereas it stimulated perilipin 2 (13). The explanation of the precise difference still remains to be investigated.

In conclusion, we found that TLR9 signals stimulate perilipin 3 expression, which leads to foam cell formation by promoting triglyceride accumulation, in conjunction with ox-LDL in macrophages. An autocrine mechanism of proinflammatory cytokines appears to be partly involved in this process. Our current study may raise a novel molecular mechanism that links TLRs to atherosclerosis formation and suggest that LDL-protein complexes could be putative molecular targets for prevention of atherosclerosis development. We expect that our results or presumptions will be verified further by animal models in which perilipin 3 is knocked down or overexpressed.

GRANTS

This work was supported in part by Grant 2004D159 from Liaoning Province Educational Office Foundation, People’s Republic of China.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


