Enhanced Plasma Levels of LIGHT in Unstable Angina
Possible Pathogenic Role in Foam Cell Formation and Thrombosis

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Background—Numerous studies have demonstrated the ability of oxidized LDL [(ox)LDL] to promote an inflammatory response in macrophages. Several inflammatory mediators have been reported to increase after oxLDL stimulation of such cells, but their relative importance is still unknown. In the present study, we used microarrays to identify genes in THP-1 macrophages that were upregulated by oxLDL.

Methods and Results—Our main findings were as follows. In a microarray screening experiment, we identified LIGHT, a ligand in the tumor necrosis factor superfamily, as one of the genes that were markedly upregulated in oxLDL-stimulated THP-1 macrophages. We showed significantly raised plasma levels of LIGHT in patients with stable angina (n=40) and particularly in those with unstable angina (n=40) compared with healthy controls (n=20), which underscores the clinical relevance of the in vitro finding. We also showed that LIGHT enhanced lipid accumulation in oxLDL-stimulated THP-1 macrophages, possibly through upregulation of class A scavenger receptor (SR-A). This increased lipid accumulation was accompanied by enhanced expression of tissue factor and plasminogen activator inhibitor-1, as well as enhanced thrombin formation, transforming macrophages into a prothrombotic phenotype. The LIGHT-mediated increase in SR-A, tissue factor, and plasminogen activator inhibitor-1 was also seen in human monocyte-derived macrophages. Finally, the LIGHT-mediated enhancement of SR-A and TF expression appears to involve nuclear factor-κB activation.

Conclusions—These findings suggest that LIGHT could serve as a molecular link between lipid metabolism, inflammation, and thrombus formation, which are all features of atherosclerotic plaques. (Circulation. 2005;112:2121-2129.)

Key Words: angina ■ inflammation ■ lipids ■ thrombosis ■ macrophage

Increasing evidence indicates that atherosclerosis is the consequence of a complex, multifactorial process that involves an abnormal interplay between lipoprotein metabolism, extracellular matrix degradation, thrombus formation, and inflammation.1-3 Exposure of macrophages to oxidatively modified LDL (oxLDL), a major component of human atherosclerotic plaques, appears to be a key event in this process, promoting both inflammation and intracellular cholesterol deposition with formation of lipid-laden foam cells.4 In addition to their role in atherogenesis, foam cells may also be involved in plaque destabilization.5 Hence, vulnerable atherosclerotic lesions are characterized by accumulation of foam cells, and destabilization of such lipid-rich areas may induce plaque rupture, leading to acute coronary syndromes.

A number of cytokines, including members of the tumor necrosis factor (TNF) superfamily (TNFSF), have been identified in atherosclerotic lesions and implicated in the pathogenesis of atherosclerosis.6-8 Both TNF-α and CD40 ligand (CD40L) appear to play pivotal roles in atherogenesis by eliciting biological responses such as secretion of inflammatory cytokines, activation of matrix metalloproteinases (MMPs), and induction of tissue factor (TF) expression.5,6,7 The list of cytokines that belong to the TNFSF has expanded significantly lately,8 and some of these "new" cytokines could also be involved in atherogenesis. Lee et al9 recently reported enhanced expression of TNFSF14, or LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for binding herpesvirus entry mediator on T cells), in human atherosclerotic plaques. These authors also showed the ability of LIGHT to induce MMP activity in macrophages. However, the role of LIGHT in atherogenesis and plaque destabilization is far from clear.

Numerous studies have demonstrated the ability of oxLDL to promote an inflammatory response in macrophages.10 Several inflammatory mediators have been reported to increase after oxLDL stimulation of such cells, but their relative importance is still unknown. In the present study, we used high-density oligonucleotide microarrays to identify genes...
regulated by oxLDL in THP-1 macrophages. These screening experiments identified LIGHT as a gene that was markedly enhanced by oxLDL. Herein, we further examined the possible pathogenic role of this oxLDL-mediated LIGHT response by several approaches, including both clinical and experimental studies.

**Methods**

**Patients and Controls**

Angina patients undergoing clinically indicated diagnostic coronary angiography in our coronary care unit were consecutively recruited to the study (Table). All patients with unstable angina (n=40) had experienced ischemic chest pain at rest within the preceding 48 hours (ie, Braunwald class IIIb) but had no evidence of myocardial necrosis by enzymatic criteria. Transient ST-T-segment depression or T-wave inversion was present in all cases. All patients with stable angina (n=40) had stable effort angina of >6 months’ duration and a positive exercise test. Exclusion criteria were myocardial infarction or thrombotic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test. Exclusion criteria were myocardial infarction or thrombolytic therapy in the previous month, ECG abnormalities, a positive exercise test.

**Cell Culture Experiments**

The human monocytic cell line THP-1 (American Type Culture Collection, Rockville, Md) was cultured in RPMI-1640 with 10% fetal calf serum (FCS; Sigma), penicillin-streptomycin, and 2 mM L-glutamine (Sigma) in 6-well trays (10^6 cells/mL, Costar) with and without different concentrations of human recombinant soluble LIGHT (Alexis), oxLDL, the thrombin receptor agonist peptide SFLLRN (100 μM/L; Biotechnology Center), or a combination of LIGHT (100 ng/mL) and oxLDL (20 μg/mL). LDL was isolated from human endotoxin-free heparin plasma and oxidatively modified by Cu^2+ ions. In some experiments, the nuclear factor (NF)-κB translocation inhibitor peptide SN50 (100 μg/mL; Calbiochem), the peroxisome proliferator-activated receptor (PPAR)-γ antagonist GW9662 (20 μM/L; Alexis), and the PPAR-γ agonist rosiglitazone (6 μM/L; Cayman Chemical) were added to cell cultures 30 minutes before LIGHT stimulation. Control cells were given vehicle (medium with solvent used for the test substances). Before all experiments, the THP-1 cells were differentiated into macrophages by incubation for 72 hours with phorbol myristate acetate (PMA; 100 nmol/mL, Sigma). Macrophage-mediated thrombin formation was assessed by an in vitro assay. For this purpose, Fragmin plasma (20 IU/mL) was prepared from healthy individuals. PMA-differentiated THP-1 macrophages (10^6 cells/mL; 0.5 mL/well; Costar), cultured in 12-well trays (Costar), were overlaid by 0.5 mL of plasma that contained either LIGHT (100 ng/mL) or vehicle and incubated for 18 hours. After incubation, samples were harvested, centrifuged at 4000g for 15 minutes, and assayed for prothrombin fragment 1+2 (F1+2) generation (see below). In experiments involving humans, monococyte-derived macrophages were isolated and cultured as described previously. After being cultured for 7 days, the cells were incubated in the THP-1 culturing medium for 12 additional hours with and without LIGHT (100 ng/mL). In all experiments, cell pellets and cell-free supernatants were stored at indicated time points at −70°C until further analysis. Endotoxin levels in all media, buffers, and stimulants were <10 pg/mL (limulus amoebocyte lysate test; BioWhittaker).

**High-Density Oligonucleotide Microarrays**

Total RNA was isolated from frozen THP-1 macrophage pellets with TRIzol (Life Technologies), quantified spectrophotometrically, and stored at −80°C. The human genome U133A array encoding 14 500 genes was purchased from Affymetrix (Santa Clara, Calif), and hybridization was performed according to the manufacturer’s protocol. Briefly, complementary DNA (cDNA) was prepared from 5 μg of total RNA. Biotin-labeled complementary RNA was obtained from in vitro transcription of cDNA and fragmented before hybridization to the array. Data analysis was performed with Affymetrix standard protocols, instrumentation, and Microarray Suite version 5.0 software.

**Real-Time Quantitative Reverse Transcription–Polymerase Chain Reaction**

Total RNA was isolated from frozen THP-1 macrophage pellets with the RNA Isolation Kit II using the MagNA Pure LC instrument (Roche Applied Science) according to the manufacturer’s description and stored at −80°C until further analysis. cDNA from total RNA was synthesized with the cDNA High Capacity Kit (Applied Biosystems). Primers were designed with Primer Express software version 2.0 (Applied Biosystems). Primer sequences can be provided upon request. mRNA was quantified with the ABI Prism 7000 (Applied Biosystems), and SyBr green assay was performed with the qPCR Master Mix for SyBr Green I (Eurogentec). The specificity of the SyBr green assays was confirmed by melting point analysis and gel electrophoresis. Gene expression of the housekeeping gene β-actin was used for normalization.

**Western Blotting**

Western blotting was performed as described previously, with separation of equal amounts of protein from each sample by SDS-PAGE (10%) before transfer onto PVDF membranes (NEN; Life Science). The membranes were incubated with antibodies against class A scavenger receptor (SR-A; Chemicon International, Temecula, Calif), followed by incubation with species-specific horseradish peroxidase–coupled secondary antibodies (Cell Signaling, Beverly, Mass). The immune complex was visualized with the Supersignal West Pico Western blot detection system (Pierce) and exposure to Hyperfilm enzyme-linked chemiluminescence (Amer sham Biosciences) and was detected with a Kodak 440 CF imaging station. The software Total Laboratory version 1.10 (Phoretx) was used for quantification. Comparisons were made only among samples transferred together onto the same membrane. To ensure equal loading, membranes were stripped and reprobed with anti-β-actin (Sigma).
Lipid Staining by Oil Red O
THP-1 macrophages were preincubated for 12 hours with LIGHT (100 ng/mL) or vehicle before a 24-hour incubation with oxLDL (20 μg/mL) in combination with LIGHT (100 ng/mL). The cells were then washed once in ice-cold PBS followed by formaldehyde fixation (2% in PBS) for 30 minutes at room temperature. Neutral lipids were stained with 0.5% oil red O (Sigma) in isopropanol for 60 minutes. The oil red O-stained lipids were measured spectrophotometrically at 492 nm after extraction.

Binding of Alexa-Labeled Acetylated LDL
Scavenger receptor binding of Alexa-labeled acetylated LDL (acLDL; AlexaFluor 488-AcLDL; Molecular Probe) in THP-1 macrophages was measured according to the manufacturer’s instruction. This fluorescence-labeled ligand binds preferentially to SR-A. Briefly, THP-1 macrophages were incubated with LIGHT (100 ng/mL) for 48 hours. The cells were chilled to 4°C in cold medium (2% BSA in RPMI-1640 with 5 mmol/L CaCl₂) for 5 minutes and washed once before 200 μL of fluorescence-labeled acLDL (10 μg/mL) in cold medium was added to each well. After 2 hours of incubation with agitation at 4°C, the medium was removed, and cells were washed 4 times with ice-cold PBS. Dyed cells were extracted in 400 μL (0.1% SDS in 0.1% NaOH) for 10 minutes at room temperature, and the ligand binding was measured with a microplate fluorescence reader (FLx800; Bio-Tek Instruments). In some parallel experiments, the slides were allowed to dry in air (dark) before examination under fluorescence microscope (Nikon Eclipse E400), and digital images with equal exposure time and magnification were made with Spot Camera RT Color (Diagnostic Instruments).

Enzyme Immunoassays
Concentrations of LIGHT were analyzed by enzyme immunoassays (EIAs) obtained from R&D Systems. F1-2 was determined by EIA obtained from Dade Behring GmbH. Cell-bound TF was analyzed by EIA (American Diagnostica) after cells were lysed completely with 1% Triton X-100.

Statistical Analysis
Data are presented as mean±SEM unless otherwise stated. For comparison of 2 groups, the Mann-Whitney U test (2 tailed) was used. When more than 2 groups were compared, the Kruskal-Wallis test was used. Probability values (2-sided) were considered significant at P<0.05.
oxLDL Induces Gene Expression of LIGHT in THP-1 Macrophages

The uptake of oxLDL by intimal macrophages, which leads to foam cell formation, is believed to play a key role in atherogenesis. To further characterize this process, we screened THP-1 macrophages for inflammatory-related genes using a microarray (see Methods) after stimulation with oxLDL (20 μg/mL) for 24 hours. Data analysis identified several genes to be upregulated at least 2-fold in oxLDL-stimulated THP-1 macrophages compared with control cells. Lee et al. have recently reported enhanced LIGHT expression in human carotid atherosclerotic plaques in regions rich in macrophages/foam cells, and notably, LIGHT was identified as one of the genes that were markedly upregulated in oxLDL-stimulated THP-1 macrophages. This oxLDL-induced effect on LIGHT mRNA level was confirmed by real-time reverse transcription–polymerase chain reaction (RT-PCR) that showed a time- and dose-dependent pattern (Figures 1A and 1B) and on the LIGHT protein level, as assessed by EIA in THP-1 macrophage supernatants after culturing with oxLDL (20 μg/mL) for up to 48 hours (Figure 1C). At the mRNA level, the most prominent effect was seen at an oxLDL concentration of 50 μg/mL (Figure 1B). However, a marked effect was also found at a concentration of 20 μg/mL, which is comparable to the plasma levels of oxLDL in CAD patients, thereby mimicking the in vivo situation, and accordingly, this concentration was used in all further experiments.

Results

Figure 3. Recombinant human soluble LIGHT stimulates SR-A expression in THP-1 macrophages. A, Effect of different concentrations of LIGHT (1 to 100 ng/mL) on mRNA levels of SR-A after culturing for up to 48 hours. The mRNA level of SR-A was normalized against gene expression of β-actin. B, Effect of LIGHT (100 ng/mL) on SR-A expression at the protein level (Western blotting). C, Representative Western blot of SR-A protein and β-actin. Data are calculated as percentages of controls (unstimulated cells) and are presented as mean±SEM of 4 to 6 independent experiments.

*P<0.05 vs controls.
Enhanced Plasma Levels of LIGHT in Patients With Stable and Unstable Angina

To elucidate the potential clinical relevance of this in vitro finding, we examined plasma levels of LIGHT in angina patients and sex- and age-matched healthy controls. As shown in Figure 2, both patients with stable angina (n=40) and particularly those with unstable disease (n=40) had significantly increased plasma levels of LIGHT compared with healthy controls (n=20). There was some overlap between angina patients and controls, which probably reflects the heterogeneity within the patient groups. A similar LIGHT pattern was also found when smokers were excluded from data analyses (data not shown), which suggests that the raised plasma levels of LIGHT in CAD do not merely reflect a nonsignificantly increased percentage of smokers in the patient groups (Table).

LIGHT Increases SR-A Expression in THP-1 Macrophages

To clarify the potential pathogenic effects of enhanced LIGHT levels in angina patients and in oxLDL-stimulated macrophages, we first examined the ability of LIGHT to modulate the expression of receptors for oxLDL in THP-1 macrophages. Although LIGHT had no effect on CD36 and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) expression (data not shown), it significantly enhanced the mRNA level of SR-A in a dose- and time-dependent manner, with a maximum increase after 12 hours (Figure 3A). Notably, we found a significantly enhancing effect of LIGHT on SR-A expression even in concentrations comparable to those detected in supernatants from oxLDL-stimulated THP-1 cells (ie, 2.5 ng/mL), which underscores the pathogenic relevance of this finding. The LIGHT-induced expression of SR-A was also confirmed at the protein level as assessed by Western blotting, which showed significantly higher protein levels both after 24 hours (≈60% increase) and after 48 hours (≈90% increase) than with controls (Figures 3B and 3C).

LIGHT Enhances Lipid Accumulation in THP-1 Macrophages

To examine any functional consequences of the enhanced SR-A expression in LIGHT-stimulated THP-1 macrophages, we next examined whether LIGHT could promote lipid accumulation in these cells. When THP-1 macrophages were costimulated with oxLDL and LIGHT, there was a significant increase in the accumulation of neutral lipids (≈20% increase, oil red O staining) compared with oxLDL stimulation alone (Figure 4A), which suggests that this cytokine could promote foam cell formation. Although this increase may appear low, it was comparable to the increase in lipid accumulation when unstimulated and oxLDL-stimulated cells were compared (Figure 4A). acLDL binds preferentially to SR-A,18 and interestingly, LIGHT enhanced the binding of fluorescence-labeled acLDL in THP-1 macrophages, which suggests that the increased foam cell formation during LIGHT stimulation is accompanied by increased “functional” expression of SR-A (Figures 4B and 4C).

LIGHT Increases Prothrombotic Mediators in THP-1 Macrophages

TF plays a central role in atherogenesis, particularly in the context of plaque instability and rupture, contributing to thrombus formation and the development of acute coronary syndromes.20 Macrophages within the arterial wall are important sources of TF,21 and to further...
examine the possible pathogenic consequences of raised LIGHT levels in stable angina and particularly in unstable angina, we investigated the effects of LIGHT on TF expression in THP-1 macrophages. As shown in Figure 5A, LIGHT significantly increased TF antigen levels both after 24 and 48 hours compared with control cells. Furthermore, in parallel samples, we found a LIGHT-mediated increase in gene expression of plasminogen activator inhibitor 1 (PAI-1), which suggests that LIGHT may also impair fibrinolytic activity, further contributing to the potential prothrombotic effects of this cytokine (Figure 5B). If such a LIGHT-stimulated enhancement of TF expression also occurs in macrophages within an atherosclerotic plaque, it could contribute to increased thrombin formation, and notably, when human plasma was added to the cell cultures of THP-1 cells (see Methods), LIGHT (100 ng/mL) significantly enhanced thrombin formation as assessed by prothrombin fragments F1+2 after culturing for 18 hours. D. Effect of the thrombin receptor agonist SFLLRN (100 μmol/L) on LIGHT mRNA levels (real-time RT-PCR) in THP-1 macrophages after incubation for various indicated time points. mRNA levels were normalized against gene expression of β-actin. Data are calculated as percentages of controls (unstimulated cells) and are presented as mean±SEM of 4 to 5 independent experiments. *P<0.05 and **P<0.01 vs controls.

Figure 5. Recombinant human soluble LIGHT increases the prothrombotic feature of THP-1 macrophages. THP-1 macrophages were incubated with LIGHT (100 ng/mL) for 24 and 48 hours before TF antigen levels in cell lysate (A) and mRNA levels of PAI-1 (B) were measured. Absolute TF concentration in unstimulated cells (100%) was 2059±208 pg/mL. When human plasma was added to the cell cultures of THP-1 cells (see Methods), LIGHT (100 ng/mL) significantly enhanced thrombin formation as assessed by prothrombin fragments F1+2 after culturing for 18 hours. D. Effect of the thrombin receptor agonist SFLLRN (100 μmol/L) on LIGHT mRNA levels (real-time RT-PCR) in THP-1 macrophages after incubation for various indicated time points. mRNA levels were normalized against gene expression of β-actin. Data are calculated as percentages of controls (unstimulated cells) and are presented as mean±SEM of 4 to 5 independent experiments. *P<0.05 and **P<0.01 vs controls.

LIGHT Increases SR-A, TF, and PAI-1 Expression in Human Monocyte-Derived Macrophages

We next examined whether the effect of LIGHT on SR-A, TF, and PAI-1 gene expression by THP-1 macrophages also could be induced in monocyte-derived macrophages from 5 healthy individuals. Notably, in these cells, we also found a LIGHT-induced upregulation of SR-A, TF, and PAI-1 expression after stimulation for 12 hours (Figures 6A through 6C, respectively). Thus, our findings in the present study appear not merely to be a phenomenon in the THP-1 cell line but also to be a characteristic of primary human macrophages.

LIGHT Modulates SR-A and TF Expression Through NF-κB Signaling and via Cross Talk With PPAR-γ Signaling Pathway

LIGHT has been found to stimulate NF-κB–dependent transcriptional activity in human hepatocytes, and a weak activation has also been found in human T cells. To investigate the mechanisms of the LIGHT-induced upregulation of SR-A and TF in THP-1 macrophages, we therefore first examined the
ability of a specific inhibitor of NF-κB translocation (SN50) to modulate this LIGHT-mediated effect. As shown in Figures 7A and 7B, in THP-1 macrophages exposed to SN50, the LIGHT enhancing effect on SR-A and TF expression was nearly abolished, which suggests the involvement of NF-κB signaling pathways.

PPARs appear to be of importance for the regulation of scavenger receptors and lipid accumulation, as well as inflammatory responses, in macrophages, and these nuclear receptors have also been suggested to have antithrombotic effects. Of the PPARs, PPAR-γ is most strongly expressed in macrophages, and we therefore next examined any possible interactions between LIGHT and this nuclear receptor in THP-1 cells. Although PPAR-γ appears to be critical for the macrophage expression of CD36, its role in SR-A regulation has been questioned. In the present study, however, we found a moderate enhancing effect of the PPAR-γ agonist rosiglitazone on SR-A expression, with an additive effect on the LIGHT-stimulated increase in this scavenger receptor (Figure 7C). In contrast, rosiglitazone inhibited both basal and LIGHT-stimulated TF protein level (Figure 7D). Finally, the PPAR-γ antagonist GW9662 had no effect on the LIGHT-induced expression of either SR-A or TF (Figures 7C and 7D). Although these findings show that the LIGHT-stimulated effect on both SR-A and TF does not involve PPAR-γ activation, our findings on the combined effects of rosiglitazone and LIGHT on SR-A and TF expression, which suggests a complex cross talk between these 2 stimulants, will have to be further elucidated.

Discussion

In the present study, we identified LIGHT as one of the genes that was markedly upregulated by oxLDL in THP-1 macrophages. We also showed significantly enhanced plasma levels of LIGHT in angina patients, with particularly high levels in those with unstable disease, which underscores the potential clinical relevance of the in vitro finding. Furthermore, we showed that LIGHT-enhanced lipid accumulation in oxLDL stimulated THP-1 macrophages, possibly via upregulation of SR-A. This increased lipid accumulation was accompanied by increased expression of TF and PAI-1 and enhanced thrombin formation, which transformed THP-1 macrophages into a prothrombotic phenotype. These findings suggest that LIGHT could serve as a molecular link between lipid metabolism, inflammation, and thrombus formation, features that all are present in atherosclerotic plaques.

TNF, lymphotoxin (LTα and LTβ), and LIGHT form a network of signaling systems involved in many aspects of immune function and inflammation. LIGHT signaling is transduced via 2 members of the TNF receptor superfamily (TNFRSF), herpesvirus entry mediator (HVEM, or TNFRSF14) and LTβR (TNFRSF3). Studies in animal models suggest that LIGHT signaling pathways may be crucial for the development of various autoimmune disorders, at least in part because of their effects on T cells (eg, increased production of interferon-γ) and T-cell homing into inflamed tissues. In the present study, we extend these findings, showing profound effects of LIGHT on THP-1 macrophages that lead to enhanced lipid accumulation and increased levels of prothrombotic mediators. Importantly, a similar LIGHT-induced pattern was also seen in human monocyte-derived macrophages. Moreover, although several studies have examined the potential pathogenic role of LIGHT in various animal models, the literature is virtually devoid of data on LIGHT in human disorders. Herein, we report high plasma levels of LIGHT in CAD patients, particularly in those with unstable disease. Together with the recent report from Lee et al9 showing increased LIGHT expression in human carotid...
Atherosclerotic plaques, the present findings suggest that LIGHT should be added to the list of inflammatory mediators that are upregulated in CAD.

A major finding in the present study was the noticeable upregulation of SR-A in LIGHT-stimulated THP-1 macrophages, as shown at both the mRNA and protein level, as well as the increased binding of acLDL to this scavenger receptor. Moreover, this LIGHT-induced upregulation of SR-A was accompanied by enhanced lipid accumulation, which suggests that LIGHT could promote foam cell formation. Modification of SR-A activity in different murine models appears to have an impact on atherosclerosis. Although the extent of the impact varies among different experimental settings, it has been shown quite convincingly that inhibition of SR-A activity reduces atherosclerosis. Interleukin-6 and interferon-γ have been reported to increase SR-A expression in macrophages, and here, we show that LIGHT is also a potent inducer of this scavenger receptor. Although LIGHT could increase foam cell formation with accumulation of oxLDL, the present findings suggest that oxLDL in and of itself enhances LIGHT expression. Interestingly, one of the LIGHT receptors (i.e., TNFRSF14/ HVEM) has been located in regions rich in macrophage-derived foam cells within human carotid atherosclerotic plaque, which suggests that such interactions between LIGHT and oxLDL, involving upregulation of SR-A, also may occur in vivo within an atherosclerotic lesion, promoting plaque progression.

Thrombosis at the site of atherosclerotic plaque disruption is the principal cause of acute coronary syndromes, and TF plays a crucial role in this process. Several members of the TNFSF have been shown to enhance TF expression in macrophages (e.g., CD40L and TNF-α), and here, we show that LIGHT also has this property, accompanied by a LIGHT-induced increase in thrombin formation as assessed by increased levels of F1+2. LIGHT was also found to increase PAI-1 expression, the major inhibitor of plasminogen activation, which further contributes to the potential prothrombotic effects of this cytokine. Moreover, although LIGHT promoted a prothrombotic phenotype in THP-1 macrophages, our findings in the present study suggest that thrombin itself could increase LIGHT expression.

Figure 7. LIGHT modulates SR-A and TF expression through NF-κB signaling and via cross talk with PPAR-γ signaling pathway. THP-1 macrophages were pretreated with the NF-κB translocation inhibitor SN50 (100 μg/mL; A and B) and the PPAR-γ agonist rosiglitazone (6 μmol/L) or the PPAR-γ antagonist GW 9662 (20 μmol/L; C and D) for 30 minutes followed by stimulation with LIGHT (100 ng/mL) for 12 hours (A and C) and 24 hours (B and D). mRNA levels were normalized against gene expression of β-actin. Absolute TF concentration in unstimulated cells (100%) was 1632 ± 86 pg/mL. Data are calculated as percentages of controls (unstimulated cells) and are presented as mean ± SEM of 4 to 8 independent experiments. *P < 0.01 and **P < 0.001 vs controls; #P < 0.01 vs LIGHT alone.
expression in these cells. Thus, the peptide SFLLRN, used as a macrophage stimulant, activates the thrombin receptor (protease-activated receptor-1) that is expressed not only on platelets but also on other cells, such as monocytes/macrophages, and thrombin and thrombin-agonists are reported to induce cytokine production in these cells. In the present study, we show that SFLLRN increases LIGHT expression in THP-1 macrophages. If operating within an atherosclerotic plaque, these interactions between thrombin formation and LIGHT expression could represent a vicious circle that contributes to inflammation and thrombus formation in CAD.

In the present study, we showed markedly increased plasma levels of LIGHT in angina patients, with particularly high levels in those with unstable disease. This raised LIGHT level could involve interactions between oxLDL and macrophages. We also showed that LIGHT could promote lipid accumulation and prothrombotic properties in these cells. Together with a previous report showing that LIGHT can induce MMP activity in THP-1 macrophages, our findings in the present study suggest that LIGHT could be involved in atherogenesis and plaque destabilization that promotes foam cell formation and thrombosis, serving as a molecular link between lipid metabolism, inflammation, and thrombus formation.

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