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LIGHT is increased in patients with coronary disease and regulates inflammatory response and lipid metabolism in oxLDL-induced THP-1 macrophages

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ABSTRACT

Inflammation is critical for the progression of hyperlipidemia. Although the exact mechanism through which inflammation affects hyperlipidemia is not very clear, evidence suggests that the tumor necrosis factor superfamily member 14 (TNFSF14/LIGHT)LIGHT might regulate lipid metabolism. In this study we investigated the expression of LIGHT in patients with different stages of coronary disease. The expression of lipid metabolism-related enzymes and inflammation-related proteins were further explored in oxidized low-density lipoproteins (oxLDL)-induced THP-1 macrophages. We found that LIGHT is highly expressed and companied with severe inflammations in patients with coronary disease. LIGHT significantly enhanced inflammation response in oxLDL-induced THP-1 macrophages. We further demonstrated that LIGHT markedly decreased the levels of lipolytic genes and increased the expressions of lipogenic genes in oxLDL-induced THP-1 macrophages. In addition, our results showed that LIGHT exerts its pro-inflammatory and pro-lipogenesis roles through activating nuclear factor-kappa B (NF-κB) signaling pathway. Taken together our study has demonstrated that LIGHT NF-κB-dependently exacerbates inflammation response and promotes lipid accumulation, and provided a new potential target for treatment of hyperlipidemia-related disease.

1. Introduction

Hyperlipidemia is characterized by abnormally elevated levels of lipids and/or lipoproteins in the blood. It is the most common cause of atherosclerosis, coronary disease and acute pancreatitis [1]. Hyperlipidemias are divided into primary and secondary subtypes. Primary hyperlipidemia is usually due to genetic causes or high fat diet, while secondary hyperlipidemia arises due to other metabolic diseases such as hypertension and diabetes. In recent years, the connection between hyperlipidemia and inflammation is attaching more and more attention. Patients with chronic infectious disease such as lupus erythematosus and rheumatoid arthritis have also showed a high incidence of hyperlipidemia [2,3]. It is reported that immune cells were involved in the regulation of lipid metabolism [4]. Similarly, hyperlipidemia is accompanied by immune activation and inflammatory response, so that atherosclerosis is considered as a lipid-driven chronic inflammatory disease. In hyperlipidemic conditions, low-density lipoproteins (LDLs) become oxidized (oxLDL) and accumulate in the arterial intima, causing an inflammatory trigger. However, the underlying mechanism by which inflammation affects lipid metabolism is not well explored.

Tumor necrosis factor superfamily (TNFSF) members play essential roles in diverse immunological processes such as T cell

Abbreviations: TNSF14/LIGHT, tumor necrosis factor superfamily member 14; oxLDL, oxidized low-density lipoproteins; NF-κB, nuclear factor-kappa B; LDLs, low-density lipoproteins; TNFSF, tumor necrosis factor superfamily; HVEM/TNFSFR14, herpes virus entry mediator; LTβR, lymphotoxin β receptor; DcR3, decoy receptor 3; APC, antigen presenting cell; SR-A, scavenger receptor-A; ACAT1, acyl-CoA: cholesterol acyltransferase-1; LTβR-Ig, LTβR immunoglobulin fusion protein; PPARα, peroxisome proliferators-activated receptor α; CPT-1, carnitine palmitoyl transterase-1; SREBP-1c, sterol regulatory element-binding protein-1c; ACS, acyl-CoA synthetase; IL, interleukin; iNOS, inducible nitric oxide synthase.

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activation, costimulation and homeostasis of immune cells [5–7]. The TNFSF member 14 (TNFSF14/LIGHT) is a type II transmembrane protein which was first identified by Dr. Mauri [8]. LIGHT assembles in homotrimers and regulates the course of the immune responses by signaling through herpes virus entry mediator (HVEM, TNFSFR14), lymphotoxin β receptor (LTβR) and decoy receptor 3 (DcR3) [9–11]. To date, LIGHT/HVEM interaction contributes to T cell activation [12,13], and LIGHT/LTβR interaction seems to be more relevant in regulating stromal/antigen presenting cell (APC)/T cell cross-talk [14]. Recently, studies in animal models and some clinical studies indicate that LIGHT may be crucial for the development of various inflammatory disorders [15,16], and it has also been implicated in the pathogenesis of vascular inflammation [17]. Furthermore, LIGHT has been associated with obesity and type 2 diabetes mellitus, potentially through promotion of inflammatory responses [18–20]. However, data on the involvement of LIGHT in atherosclerosis are scarce.

Based on the role of LIGHT in vascular inflammation and metabolic disorders, we assumed that LIGHT could be involved in the pathogenesis of atherosclerosis, which is a typical example of hyperlipidemia. This hypothesis was investigated by various experimental approaches, including clinical studies in patients with coronary disease and experimental studies in oxLDL-induced human THP-1 macrophages.

2. Materials and methods

2.1. Patients

Thirty-two patients with coronary disease were divided into two groups (unstable angina pectoris and stable angina pectoris) according to the clinically diagnostic criteria. Additional 16 healthy people were served as normal control. Blood samples from these volunteers were collected. All patients were recruited from the Cardiology Department of Sichuan Provincial People's Hospital (Chengdu, China). The study was approved by the Ethics Committee of our institution. Informed consent was signed by the participants.

2.2. Enzyme-linked immunosorbent assay (ELISA)

ELISA assay was performed with specific antibody according to the manufacturers' instructions (Sigma, St. Louis, USA). Plasma samples were precoated onto ELISA plates and served as the antigen. o-Dianisidine was used as substrate and the absorbance of the colored horseradish peroxidase (HRP) product was measured spectrophotometrically at 405 nm by an automated microplate reader (Thermo, Waltham, USA).

2.3. Flow cytometry

The percentage of $CD68^+$ or $CD163^+$ cells was detected by flow cytometry. Briefly, plasma samples were incubated with ionomycin, phorbol ester, and monensin (Sigma, San Francisco, USA) for 4 h at 37 °C. Cells were then stained with FITC-labeled anti-CD68 and anti-CD163 (BD Biosciences, San Diego, USA), respectively, for 30 min at room temperature. Data acquisition was performed on a BD-LSR II (BD Biosciences, San Diego, CA), and the data were analyzed with FlowJo software.

2.4. Cell culture and treatment

THP-1 cells was maintained routinely in serum-free RPMI Media 1640 (Gibco, Grand Island, NY) and grown at 37 °C in a 5% CO_2 atmosphere. Cells were differentiated into macrophages by stimulation of phorbol ester (PMA, 100 ng/ml; Sigma, St. Louis, USA) and then incubated with ox-LDL (20 mg/L; Sigma, St. Louis, USA) only or added with recombinant human LIGHT (200 ng/ml; R&D Systems, Minneapolis, Minneapolis, USA) for 24 h.

2.5. Western blot

Total protein samples from cells were prepared with standard protocol. Equivalent amounts of protein samples were separated by 10% SDS-PAGE and transferred to PVDF membranes (Millipore, Billerica, USA). Membranes were then incubated at room

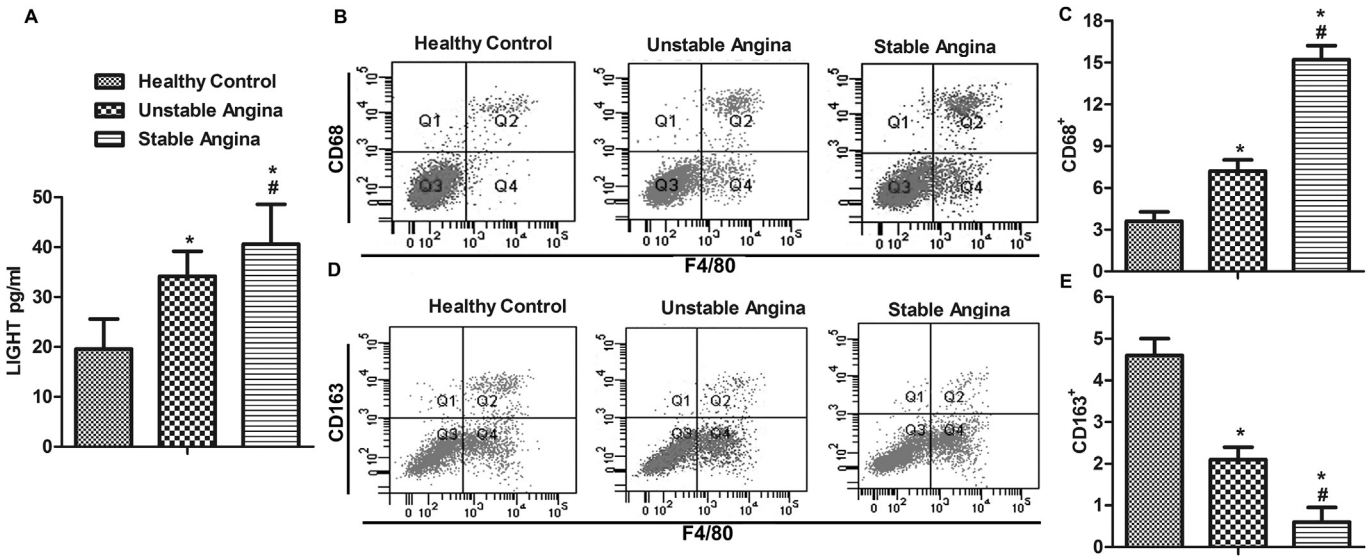

Fig. 1. Patients with coronary disease display high expression of LIGHT and high level of inflammatory reaction. (A) Patients with coronary disease were divided into two groups: unstable angina pectoris and stable angina pectoris. Healthy volunteers were taken as control group. The expression of LIGHT in plasma was detected by ELISA. (B) The $CD68^+$ cells were separated with a flow cytometer. (C) Quantification of Fig.1B. (D) The $CD163^+$ cells were separated with a flow cytometer. (E) Quantification of Fig.1D. All the experiments were repeated at least three times. $^*P < 0.05$ versus healthy control, $^\#P < 0.05$ versus Unstable Angina.

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