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Atherogenic effects of TNF-α and IL-6 via up-regulation of scavenger receptors

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ABSTRACT

Patients with chronic inflammatory disorders such as rheumatoid arthritis (RA) have a high risk of developing cardiovascular disease. We evaluated the effects of TNF-α and IL-6 on foam cell formation, a pivotal process in atherogenesis. Accumulation of intracellular oxidized LDL (oxLDL) was induced when THP-1/macrophages were stimulated with TNF-α or IL-6. TNF-α induced the expressions of scavenger receptors SR-A and LOX-1, and IL-6 induced SR-A expression. Inhibition of the NF-κB signaling markedly decreased TNF-α-induced foam cell formation and SR-A expression. Serum from RA patients, but not healthy subjects, induced foam cell formation, which was partially reversed by either IL-6 or TNF-α blockade in conjunction with inhibiting the induction of scavenger receptors. The present study clearly showed that in patients with chronic inflammation mediated by TNF-α and IL-6, these cytokines are directly implicated in atherosclerotic plaque formation.

1. Introduction

In patients with chronic inflammatory diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus, the risk of cardiovascular disease (CVD) is increased [1,2]. Inflammatory cytokines are considered of fundamental importance in the development of CVD. The greatest advance in the therapy of RA has been the identification of the key pathogenic roles played by TNF-α and IL-6. Interestingly, one recent study showed that TNF-α blockade also has a beneficial effect on the risk for mortality from CVD in RA patients [3]. However, the mechanisms underlying this preventive effect of TNF-α blockade on CVD are not yet fully clear. Mortality due to CVD is increased in inflammatory diseases [4,5], but this elevated risk is not explained by traditional risk factors for CVD such as elevated LDL cholesterol, smoking, high fat diet, or heredity [6–8]. Paradoxically, although TNF-α blockade therapy significantly increases serum levels of cholesterol and LDL, it nevertheless decreases the onset of CVD.

The pathway leading to atherosclerotic plaque and cardiovascular events begins with fatty streak formation and macrophage infiltration. Macrophages are dedicated phagocytes and their sole function is to remove small particles like lipoproteins as well as pathogens and dead or damaged cells. After phagocytosis, lipoproteins are efficiently delivered to lysozymes for degradation [9]. However, oxidized low density lipoprotein (oxLDL) protein is resistant to lysosomal degradation and accumulates along with oxLDL cholesterol [10]. This accumulation results in the transformation of macrophages into foam cells, resulting in the formation of plaque. oxLDL and its scavenger receptors participate in all of these processes. Therefore, in this study, we evaluated the effect of TNF-α and IL-6 on the pivotal process of oxLDL-mediated atherogenesis, and thus, foam cell formation.

We demonstrated that TNF-α and IL-6 were each able to promote oxLDL accumulation and foam cell formation by up-regulating the expressions of scavenger receptors: human scavenger receptor-A (SR-A) and lectin-like oxidized LDL receptor-1 (LOX-1). In addition, IL-6 induced by TNF-α at least partially mediated the foam cell formation by TNF-α. Finally, we found that foam cell formation and the expression of scavenger receptors were significantly augmented by the serum of RA patients, but not by the serum of healthy subjects.

2. Material and methods

2.1. Reagents and cells

A humanized anti-human IL-6R antibody (tocilizumab, αIL-6R Ab, [11]) was prepared in our laboratories. Recombinant human TNF-α and human IL-6 were purchased from PeproTech (Rocky Hill, NJ, USA). A recombinant TNF-α-receptor (p75)-Fc-fusion protein (etanercept, αTNF-α Ab) was purchased from Wyeth (Munster, Germany). Human IgG and recombinant human IFN-γ were purchased from Sigma–Aldrich (St Louis, MO, USA). The human monocyte cell line THP-1 was obtained from ATCC (Manassas, VA, USA) and cultured using RPMI-1640 medium with 10% FBS.

Serum samples of 10 RA patients and 10 healthy subjects were obtained from ProteoGenex (Culver City, CA, USA). Prior to

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participation, written informed consent was obtained from all subjects. The study was performed in accordance with the Declaration of Helsinki and the experimental protocol was approved by the committee of Chugai Pharmaceutical Co., Ltd. Patient information (e.g. age, sex, disease duration, other disease, prescription drugs) was obtained from ProteoGenex. TNF-α, IL-6, and IFN-γ concentrations of serum samples were measured with a Quantikine high sensitivity ELISA kit (R&D Systems, Minneapolis, MN, USA).

2.2. Foam cell formation induced by cytokines and serum

THP-1 cells (1×10^6 cells/mL) were cultured with 100 ng/mL of phorbol myristate acetate (PMA; Sigma–Aldrich) in 12- or 48-well plates for 4 days at 37 °C to induce them to differentiate into macrophage-like cells (THP-1/macrophages). THP-1/macrophages were washed twice with PBS and were then incubated for 24 h with IFN-γ (10 ng/mL), IL-6 (0.1–10 ng/mL), TNF-α (0.1–10 ng/mL), or serum samples in the presence or absence of human IgG (10 μg/mL), αIL-6R Ab (10 μg/mL), or αTNF-α Ab (10 μg/mL). Serum was diluted with medium (serum:medium = 1:3). Cell supernatants were collected for cytokine measurement. For mRNA measurement, total RNA was extracted using an RNeasy kit (Qiagen, Valencia, CA, USA).

Cells were further incubated with oxLDL (Intracel, Frederick, MD, USA; 100 μg/mL) for 48 h. After incubation, the medium was aspirated and the remaining cells were fixed with 4% formaldehyde for 2 min. After that, cells were stained with Oil Red O stain (Diagnostic BioSystems, Pleasanton, CA, USA) for 6 min. Lipid accumulation was observed.

2.3. Dil-oxLDL uptake assay

The uptake of fluorescently labeled oxLDL (labeled with 1,1′-dioctadecyl-3,3,3′3′-tetramethylindocarbocyanine perchlorate [Dil]) was determined to detect foam cell formation. THP-1/macrophages were incubated with IL-6 or TNF-α in the presence or absence of human IgG, αIL-6R Ab, or αTNF-α Ab for 24 h. THP-1/macrophages were incubated in a medium containing 10 μg/mL Dil–oxLDL (Biomedical Technologies, Stoughton, MA, USA) for a further 4 h, washed 5 times with PBS, and then extracted with iso-propranolol as previously reported [12]. The uptake of Dil-oxLDL was quantified by measuring the fluorescence for each sample (520/564 nm), with results expressed in arbitrary units of fluorescence intensity.

2.4. Measurement of TNF-α and IL-6

TNF-α and IL-6 concentrations in the supernatants were measured by ELISA kit (Bender Medsystems GmbH, Vienna, Austria;

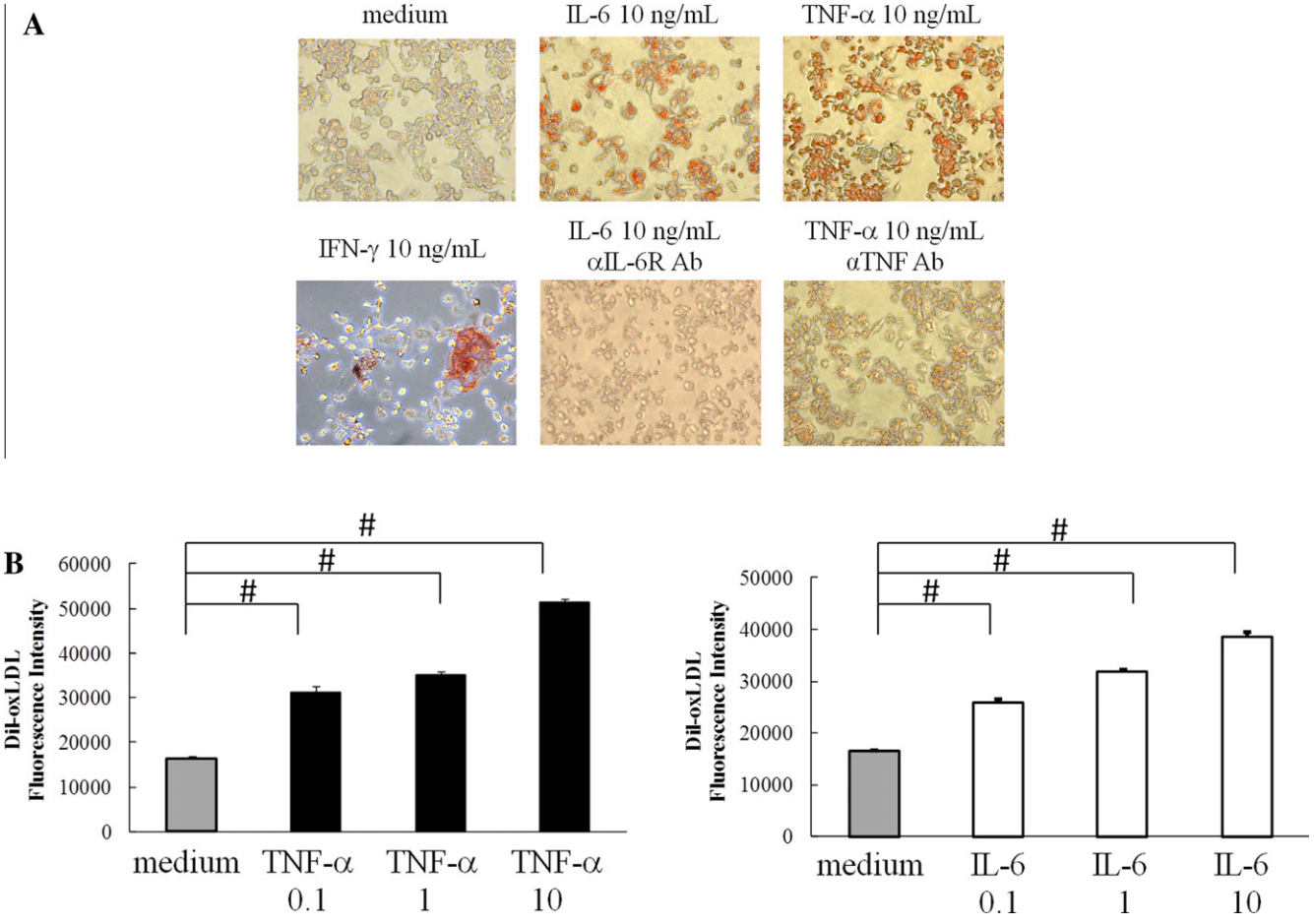

Fig. 1. The effect of TNF-α and IL-6 on oxLDL uptake into macrophages THP-1/macrophages were incubated for 24 h with IFN-γ (10 ng/mL), IL-6 (0.1–10 ng/mL), or TNF-α (0.1–10 ng/mL). Cells were further incubated with oxLDL or Dil-oxLDL for 48 or 4 h, respectively. (A) Intracellular oxLDL accumulation was observed in THP-1/macrophages stimulated with IFN-γ, TNF-α, IL-6, αTNF-α Ab (10 μg/mL) and αIL-6R Ab (10 μg/mL). (B) The uptake of Dil-oxLDL was quantified by measuring the fluorescence emission for each sample. Results are expressed in arbitrary units of fluorescence intensity. Total RNA was collected after 24 h incubation with TNF-α (0.1–10 ng/mL) or IL-6 (0.1–10 ng/mL). Each column and vertical line represents the mean and SD of at least triplicate cultures. $^{\#}p < 0.05$, Dunnett's multiple comparison test. Experiments were repeated at least 3 times.