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Interleukin-10 down-regulates oxLDL induced expression of scavenger receptor A and Bak-1 in macrophages derived from THP-1 cells

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ABSTRACT

Here, we investigated the therapeutic potential of IL-10 by testing its effects on oxLDL-induced lipoprotein uptake and apoptosis by flow cytometry in THP-1-derived macrophages. The mRNA and protein expressions of lipid scavenger receptors (SR-A, CD36) and apoptosis-related proteins (Bcl-2, Bak-1) were also detected. Co-incubation of oxLDL with IL-10 reduced DiI-oxLDL uptake by 16.1 ± 3.8%, 35.2 ± 3.8% and 28.9 ± 1.8% at 6, 12 and 24 h of treatment, respectively. Furthermore, treatment with oxLDL for 24 h enhanced the SR-A mRNA and protein expressions by 89.3 ± 17.1% and 70.1 ± 17.6%, respectively. IL-10 abrogated the oxLDL-induced SR-A mRNA expression by 50.2 ± 3.9% and its protein by 45.6 ± 1.9%. Meanwhile IL-10 had no effect on the oxLDL-induced increase of CD36 expression. IL-10 inhibited the oxLDL-induced cell apoptosis in a time-dependent manner by 17.3 ± 3.3%, 36.4 ± 2.8% and 31.0 ± 4.3% at 6, 12 and 24 h, respectively. OxLDL increased Bak-1 mRNA and protein expressions by 38.4 ± 13.3% and 36.9 ± 12.1%, respectively. However co-stimulation of oxLDL with IL-10 for 24 h inhibited Bak-1 expression to 28.4 ± 7.2% (mRNA) and 25.7 ± 6.3% (protein). Meanwhile, IL-10 had no effect on the oxLDL-induced decrease of Bcl-2 expression. Our findings suggested that IL-10 reduced the oxLDL-induced lipoprotein uptake and apoptosis partly via down-regulating the oxLDL-induced expression of SR-A and Bak-1 in THP-1-derived macrophages.

Introduction

Atherosclerosis is a chronic arterial disease with life-threatening complications, including myocardial and cerebral infarcts, which occur with increased frequency and greater severity in younger adults [1]. It is a multi-factorial disease with elements of both lipid deposition and inflammation [2]. In an inflammatory environment provoked by the presence of oxidized low density lipoprotein (oxLDL)[1], activated macrophages are able to digest lipid via scavenger receptors (SR) on the surface of cells [3]. SR-A and CD36 have been shown to be responsible for the high proportion of modified LDL uptake in macrophages, and other scavenger receptors cannot compensate in their absence [4]. In contrast to most cytokines that act as strong paracrine amplifiers of the immune-inflammatory process in the vessel wall, IL-10 secreted by activated monocytes/macrophages and T-cells has pronounced immunosuppressive and anti-inflammatory effects. The inflammatory component of atherosclerosis therefore offers a promising target for IL-10, which has therapeutic potential in various inflammatory diseases [5]. Clinical studies have also revealed that high serum levels of IL-10 in patients with acute coronary syndromes have better clinical outcomes [6].

Our previous *in vitro* study found that IL-10 is able to inhibit foam cell formation [7]. However, the mechanisms underlying the inhibitory effects of IL-10 on the lipid deposition and foam cell formation are still unclear. Recent studies have speculated that de-regulation of apoptosis occurs in atherosclerotic lesions [8]. The imbalance between cell survival and death may contribute to dramatic alterations in cellularity of the arterial wall with atherosclerosis. OxLDL is known that to induce apoptosis in macrophages [9], and SR-A can regulate apoptosis in these cells [10]. Meanwhile, IL-10 has anti-apoptotic properties in various cell types [11].

The Bcl-2 family of proteins is characterized by its ability to modulate cellular apoptosis under a broad range of physiologic conditions. Bcl-2 is one of many key regulators of apoptosis which are essential for proper development, tissue homeostasis and protection against foreign pathogens. Human Bcl-2 is a membrane-associated, anti-apoptotic oncoprotein that can promote cell

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[1] IL-10, interleukin-10; oxLDL, oxidized low-density lipoprotein; DiI-oxLDL, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine (DiI)-labeled oxLDL; SR-A, scavenger receptor-A; Bcl-2, B cell lymphoma/lekmia-2; Bak-1, Bcl-2 homologous antagonist/killer-1.

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survival through protein–protein interactions with other Bcl-2 related family members, such as the death suppressors Bcl-xL, Mcl-1, Bcl-w and A1, or the death agonists Bax, Bak, Bik, Bad and BID [12]. One member of the Bcl-2 family, Bak, functions primarily to enhance apoptotic cell death following appropriate activating signals and to counteract the protective effect provided by Bcl-2. Additionally, the pro-apoptotic activity of Bak-1 is broadly distributed [13].

In this paper, we investigated the potential ability of IL-10 to modulate oxLDL uptake and oxLDL induced apoptosis in macrophages. Specifically, the effects of IL-10 on uptake of lipoproteins, expression of key receptors (SR-A and CD36), apoptosis and the expression of apoptotic factors Bcl-2 and Bak-1 in oxLDL treated macrophages derived from the THP-1 human acute monocytic leukemia cell line.

Materials and methods

Cell and treatment groups

The THP-1 cell line was obtained from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 200 U/ml penicillin G and 200 μg/ml streptomycin. Prior to all experiments, the THP-1 cells were differentiated into macrophages by incubation with 200 nM PMA (phorbol myristate acetate, Sigma, St. Louis, USA) for 24 h [14]. Thereafter, four groups of the differentiated macrophages were set up and further incubated with or without 20 μg /L IL-10 (R&D, Minneapolis, USA,) for 24 h or with 100 mg/L oxLDL (Yuanyuan Biotechnology, Guangzhou, China) in the presence or absence of 20 μg/L IL-10 for 24 h. The oxLDL levels were measured by the TBA method using an MDA (maleic dialdehyde) Kit, and the degree of oxidation was calculated to be more than 50%. In addition the purity of the oxLDL was tested using the MTT method to ensure that it was free of contaminating toxins prior to use in the experiments [7]. The optimal dose and incubation time for the response to oxLDL or IL-10 were determined in previous experiments [7,15].

Lipoprotein uptake assay

For the lipoprotein uptake assay, DiI-oxLDL (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine-labeled oxLDL, Yuanyuan Biotechnology, Guangzhou, China) was used as the ligand. The macrophages were pre-incubated with 20 mg/L DiI-oxLDL [16] in RPMI 1640 medium containing 10% FBS at 37 °C in the presence or absence of IL-10 20 μg/L for 6, 12 and 24 h. At the end of incubation period, the cells were washed twice with ice-cold PBS, harvested by trypsinization and suspended at a concentration of 1×10^6 cells/mL in 2% FBS/PBS. The fluorescent cells were analyzed by flow cytometry (BD FACSCalibur, BD Biosciences, USA) using Flowjoe software, and 1×10^4 individual live cells were determined for each sample.

Apoptosis and cell death assay

Apoptosis in the cells of the four treatment groups were evaluated at the 6, 12 and 24 h time points. Annexin V-APC (eBioscience, San Diego, USA) conjugated with the vital dye propidium iodide (PI) was used to distinguish early stage apoptotic cells (annexin+/PI−) from necrotic cells (annexin−/PI+) or those cells that had already died due to apoptosis (annexin+/PI+). The detail protocol was provided by the kit. Uptake of the fluorescent dyes was measured in 1×10^4 cells by flow cytometry (BD FACSCalibur, BD Biosciences, USA) and the collected data were analyzed using Flowjoe software. Cells treated with H_2O_2 (50 μmol/L) served as a positive control for the experiment.

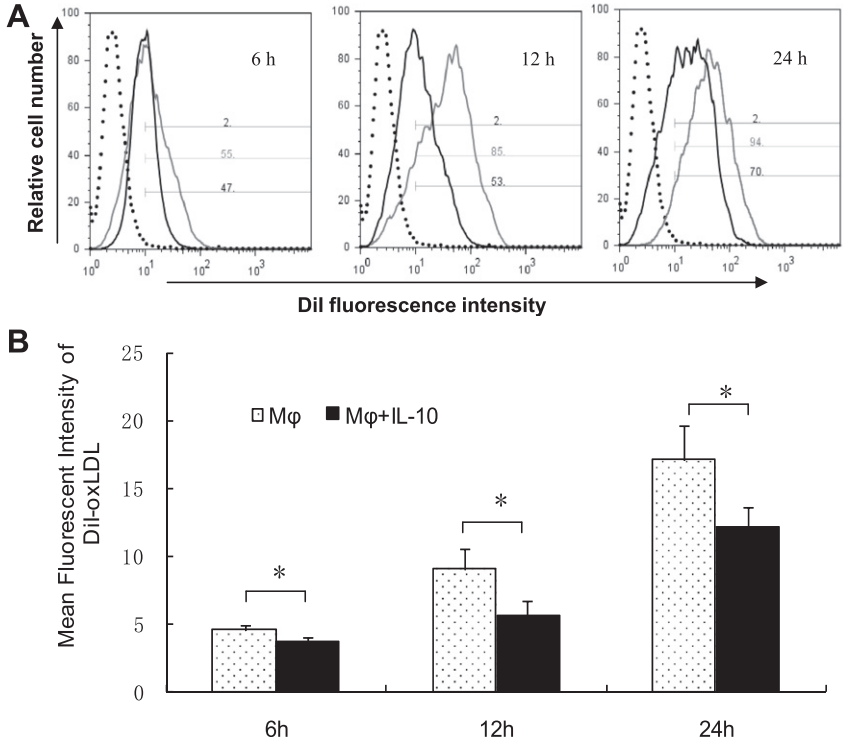

Fig. 1. The time-dependent effect of IL-10 on lipid uptake in THP-1-derived macrophages. The cells were stimulated with 20 mg/L DiI-oxLDL in the presence or absence of 20 μg/L IL-10 for 6, 12 and 24 h. The levels of DiI-oxLDL taken into the cells were analyzed by flow cytometry. (A) Typical histogram profiles of DiI-oxLDL uptake over the time course. The numbers of positive cells were distributed differently in the cells treated with DiI-oxLDL (gray line), DiI-oxLDL plus IL-10 (dark line), and untreated cells (Dot-line). (B) The relative MFI over the time course are shown as means ± S.E.M. from three separate experiments. Asterisks (*) indicate $P < 0.05$ (* vs. control group).