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Anti-obesity and anti-inflammatory effects of macrophage-targeted interleukin-10-conjugated liposomes in obese mice

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ABSTRACT

Obesity is associated with chronic inflammation and is known as a major risk factor for several diseases including chronic kidney disease, diabetes, and cardiovascular diseases. Macrophages play a critical role in the development of obesity-induced inflammation. Efficient delivery of therapeutic anti-inflammatory molecules, such as interleukin (IL)-10, to macrophages can dramatically improve therapeutic efficacy of obesity treatments. We used liposomes containing the 'eat-me' signal phosphatidylserine (PS) (PS-containing liposomes; PSL), which have macrophage targeting ability and anti-inflammatory functions, as a biomaterial carrier for the delivery of IL-10 to macrophages. The IL-10-conjugated PSL (PSL-IL10) showed high affinity for macrophages. In obese mice, PSL-IL10 treatment exhibited significant anti-obesity and anti-inflammatory effects, such as reduced serum total cholesterol, adipocyte size, crown-like structures, proinflammatory cytokine secretion (IL-6 and tumor necrosis factor α) in adipose tissue, liver injury, hepatic steatosis, and inflammation foci, while treatment with IL-10 or PSL alone did not. These findings suggest that the PSL-IL10 has macrophage targeting ability and enhanced anti-inflammatory effect due to the synergistic anti-inflammatory effects of IL-10 and PSL, and can be used as a macrophage-targeted therapeutic material for inflammation-related diseases, including obesity.
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1. Introduction

Obesity is a risk factor for several diseases, including chronic kidney disease [1], non-alcoholic fatty liver disease [2], type 2 diabetes [3,4], and cardiovascular diseases [5,6]. It is also associated with a state of chronic systemic low-grade inflammation. Inflammatory processes stimulated by obesity increase circulating levels of proinflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α [4,7].

Macrophages play a critical role in the development of obesity-induced inflammation. Macrophage infiltration is elevated in the adipose tissue (AT) of obese compared with lean individuals or animals. AT macrophages (ATMs) are derived mainly from blood monocyte-derived macrophages, and their recruitment to ATs is enhanced in the presence of obesity-induced inflammation [8]. There is a significant relationship between ATM number and inflammatory response [8–10]. These results suggest that ATMs are the primary inflammatory source in AT and play a role in the obesity-related inflammatory response. In fact, obesity induces inflammatory M1 macrophage infiltration and inflammatory cytokine secretion in ATs [9–11]. In contrast, an increase in anti-inflammatory M2 macrophages in ATs upregulates the expression of anti-inflammatory cytokine IL-10 and protects adipocytes from TNF-α-induced insulin resistance [10,12].

The anti-inflammatory cytokine IL-10 is produced by various cells of the innate and adaptive immune system, including dendritic cells, natural killer cells, eosinophils, neutrophils, monocytes, macrophages, B cells, mast cells, and all T cell subsets (Th1, Th2, Th9, and Th17 effector T cells, regulatory T cells, and $CD8^+$ T cells), but its major source is macrophages [13,14]. IL-10 reduces the production of pro-inflammatory cytokines in macrophages through the STAT3-dependent pathway [15,16]. Administration of IL-10 is effective for

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preventing and treating several inflammatory and autoimmune diseases, such as psoriasis, inflammatory bowel disease, allergic contact dermatitis, and systemic lupus erythematosus [13,14]. IL-10 stimulates anti-inflammatory signaling pathways through its interaction with the IL-10 receptor, and IL-10 receptor deficiency on macrophages can lead to severe inflammatory responses and marked proinflammatory cytokine production [17,18]. In contrast, safe and efficient delivery of therapeutic molecules (drugs, genes, and proteins) into targeted cells can dramatically improve therapeutic efficacy [19–21]. Given that IL-10 has a very short half-life [22,23], its efficient delivery to macrophages may increase anti-obesity and anti-inflammatory effects in obesity therapy.

In the present study, liposomes containing the 'eat me' signal phosphatidylserine (PS) were used as a biomaterial carrier for the delivery of IL-10 to macrophages. Macrophages can specifically recognize PS on apoptotic cells and the PS-dependent phagocytosis of apoptotic cells by macrophages can inhibit proinflammatory cytokine production. PS-containing liposomes (PSLs) mimic apoptotic cells, and can change inflammatory M1 macrophages to anti-inflammatory M2 macrophages [24–26]. Previous studies suggested that nanoparticles containing PS are useful for the alleviation or treatment of inflammation-related diseases, such as myocardial infarction [27]. rheumatoid arthritis [28]. retinal ischemia-reperfusion injury [29]. and atopic dermatitis [30]. Therefore, the conjugation of IL-10 to PSL may result in increased macrophage targeting ability and enhanced anti-inflammatory effect in obesity treatment due to the synergistic anti-inflammatory effects of IL-10 and PSL.

The purpose of this study was to investigate whether the IL-10-conjugated PSL (hereafter referred to as PSL-IL10) can reduce obesity-related and inflammatory parameters in a high-fat diet (HFD)-induced obesity mouse model.

2. Materials and methods

2.1. Synthesis of PSL and PSL-IL10

Phosphatidylserine (PS) with C18:0 alkyl groups (purity ≥98%) and phosphatidylcholine (PC) with C16:0-C18:2 alkyl groups (purity ≥98%) (all Sigma-Aldrich, St. Louis, MO, USA) were dissolved in chloroform/methanol (90:10, v/v). PSL was prepared from a lipid mixture of PS (14 mM) and PC (33 mM) at a molar ratio of 3:7 and PC liposome (PCL) from PC only, with or without the fluorescent dye [1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(carboxyfluorescein) (ammonium salt)] (Avanti Polar Lipids, Inc., Alabaster, Alabama, USA). The amount of fluorescent dye was 14.6 μg per mg of liposome. The solvent was removed in a rotary evaporator at 30 °C under reduced pressure, then dried in a desiccator for 2 h, and resuspended in PBS (10 mg/ml).

Recombinant mouse IL-10 (BioLegend, San Diego, CA, USA) was derivatized with *N*-hydroxysuccinimide ester of palmitic acid (purity ≥98%) (Sigma-Aldrich) as previously described with modifications [31]. The *N*-hydroxysuccinimide ester of palmitic acid was dissolved in ethanol at 10 mg/ml and heated to 50 °C. Exactly 10 μl of the solution was added to the preheated IL-10 solution to 37 °C and the mixture was stirred at 37 °C for 6 h. The lipid-derivatized IL-10 was purified using Sephadex G-25 column (GE Healthcare Bio-Science, Tokyo, Japan) and was adjusted to 0.1 mg/ml by measuring absorbance at 280 nm according to standard curves. PSL-IL10 was prepared by mixing equal volumes of PSL solution (10 mg/ml) and the lipid-derivatized IL-10 solution (10 μg/ml) for 20 min at room temperature. The unconjugated IL-10 was removed by ultracentrifugation twice at 100,000 g for 60 min at 4 °C. The amount of IL-10 conjugated to liposomes was analyzed by the microassay of Bradford method (Coomassie Brilliant Blue G-250 reagent; BIO-RAD Lab., Hercules, CA, USA) and was 0.86 ± 0.02 μg per mg of liposome.

2.2. Measurement of diameter and zeta-potential of PSL-IL10 and PSL

Milli-Q water (900 μl; pH 7.3) was added to PSL or PSL-IL10 solution (each 100 μl). The diameter and zeta-potential of samples were determined using a Zetasizer (Malvern Instruments, Malvern, UK) with a helium/neon (He/Ne) laser at a detection angle of 173° at 25 °C.

2.3. In vitro experiments

Raw 264.7 cells were maintained in Iscove's Modified

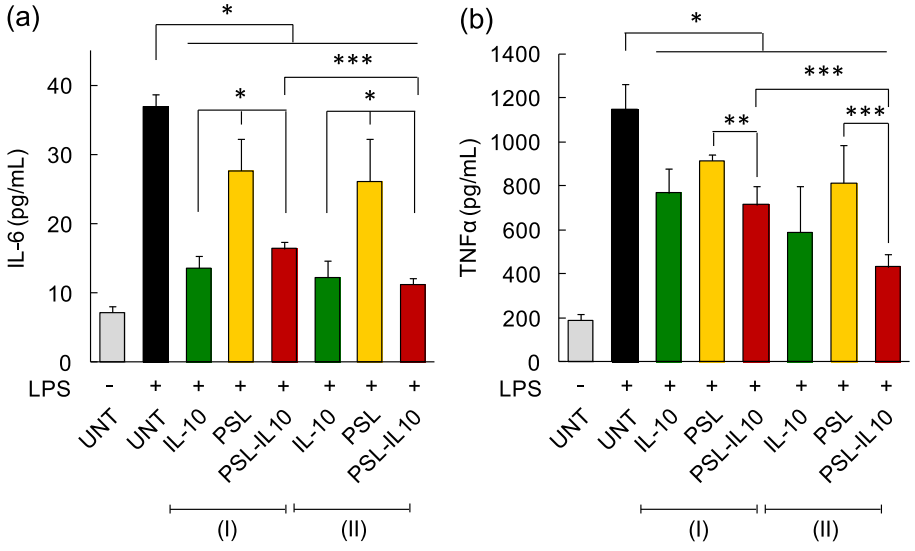

Fig. 1. Anti-inflammatory effects of PSL-IL10 and PSL. The levels of inflammatory cytokines, a) IL-6 and b) TNF-α, were determined at 24 h after adding PSL-IL10 and PSL to Raw 264.7 cells stimulated with LPS (1 μg/ml). (I) IL-10 (3 ng/ml), PSL (3 μg/ml), PSL-IL10 [IL-10 (3 ng/ml)-conjugated PSL (3 μg/ml)]. (II) IL-10 (10 ng/ml), PSL (10 μg/ml), PSL-IL10 [IL-10 (10 ng/ml)-grafted PSL (10 μg/ml)]. UNT, untreated. $n = 4$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$; one-way ANOVA with two-tailed Student's *t*-test.

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