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Cytokine-mediated modulation of leptin and adiponectin secretion during in vitro adipogenesis: Evidence that tumor necrosis factor-α- and interleukin-1β-treated human preadipocytes are potent leptin producers

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Abstract

Over the last decade, compelling evidence has been presented that cytokines affect adipocyte tissue formation and function. In this study we explored the effect of pro-inflammatory (i.e. interleukin (IL)-1β, IL-6, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α) versus anti-inflammatory cytokines (i.e. IL-4, IL-10, and transforming growth factor (TGF)-β_1) on leptin and adiponectin secretion during in vitro human adipogenesis. Confirmative to previous reports, conversion of precursor preadipocytes into mature adipocytes was completely inhibited upon exposure to TNF-α, IL-1β, IFN-γ, or TGF-β_1. Hence, all these anti-adipogenic cytokines prevented release of adipocyte-specific adiponectin. IFN-γ also strongly reduced leptin production (≥85%). However, TNF-α, IL-1β, and TGF-β_1 stimulated leptin production from preadipocytes in the absence of mature adipocytes (20.6 ± 5.4 ng/ml, 100.8 ± 18.2 ng/ml, and 5.4 ± 0.4 ng/ml, respectively, compared to 6.6 ± 0.8 ng/ml in control adipocyte cultures on day 21; $n = 4$). IL-4, IL-6 and IL-10 did not, or only slightly, affect adipocyte differentiation and their hormonal secretion. In conclusion, adiponectin and leptin are both synthesized by adipocytes, whereas leptin is also produced by preadipocytes upon TNF-α or IL-1β stimulation. These data suggest that preadipocytes could contribute more to total circulating leptin levels than has been previously considered, especially in diseased conditions were these pro-inflammatory factors play a prominent role.

Keywords: Adipocyte; Adiponectin; Cytokine; Leptin; Preadipocyte

1. Introduction

Adipose tissue, apart from being a lipid-storing organ, produces and secretes an array of bioactive molecules, also called 'adipokines', including adiponectin, leptin, resistin, plasminogen activator inhibitor-1, and cytokines like tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β and interleukin (IL)-6 [1].

Leptin is synthesized by adipocytes and is an important regulator of energy balance through its action in the brain on appetite and energy expenditure [2]. In addition to this role in energy homeostasis, leptin is also known for its effect on the immune system [3,4]. Impaired immunological responses occur in humans during starvation and malnutrition, two conditions characterized by low levels of circulating leptin levels [5,6]. In normal mice, leptin inoculation corrected starvation-induced immunological deficits [7]. In addition, in vitro

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macrophage functions (e.g. phagocytosis and lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines) were enhanced by leptin exposure [8]. Leptin also augmented proliferation and functional activation of myelocytic and primitive hematopoietic progenitor cells [9,10], and modulated CD4 T lymphocytes activation towards T helper type 1 (Th_1) phenotype by stimulating the synthesis of interferon (IFN)-γ and IL-2 [11]. In contrast, leptin demonstrated anti-inflammatory action on peripheral blood mononuclear cells from relapsing multiple sclerosis patients by suppression of IFN-γ and induction of IL-10 production [12]. Recently, the in vivo immunological role of leptin has been proved by the beneficial effect of leptin on T-cell hyporesponsiveness in human congenital leptin deficiency [13]. Altogether, these findings clearly indicate that leptin is an immunostimulatory molecule.

Adiponectin (also called Arcp30, GBP28, apM1 or AdipoQ) is produced by adipocytes [14], and plays an insulin-sensitizing role in glucose and lipid metabolism of adipocytes, skeletal myocytes, and hepatocytes [15]. Besides this endocrine function, adiponectin has recently been shown to display anti-atherogenic and anti-inflammatory properties: adiponectin inhibited the transformation of macrophages to become atherosclerotic foam cells [16], and suppressed phagocytosis and LPS-induced TNF-α or IFN-γ production by mature macrophages [17,18]. However, anti-inflammatory factors, like IL-10 and IL-1 receptor antagonist (IL-1RA), were augmented in adiponectin-treated macrophages [18]. Furthermore, adiponectin hampered proliferation of myelomonocytic lineage cells and of oxidized low-density lipoprotein-treated endothelial cells [19,20], and decreased expression of various adhesion molecules on TNF-α-treated aortic endothelial cells, thereby preventing monocyte attachment [20]. These observations strongly suggest that adiponectin acts as an important suppressive regulator of inflammatory immunological responses, especially in endothelial cells and macrophages. This concept is, although circumstantially, supported by the observations that adiponectin blood levels in patients with atherosclerosis or with obesity-associated coronary artery disease and type 2 diabetes were found to be lower in comparison to healthy individuals [21–23]. Furthermore, in apolipoprotein E-deficient mice, a well-established experimental model to study atherosclerosis development, adiponectin administration significantly reduced aortic lesion formation [24].

Alongside the above-described impact of leptin and adiponectin on the immune system, pro-inflammatory (e.g. TNF-α and IL-6) cytokines reciprocally affect the adipocyte/endocrine system [25]. These cytokines, either produced by adipocyte tissue itself or by (non)immune cells, were primarily investigated because of their assumed role in the development of vascular complications and diabetes related to obesity [26,27]. In general, pro-inflammatory cytokines have been demonstrated to prevent in vitro adipocyte conversion from preadipocytes and to enhance lipolytic activity in adipocytes, leading to so-called de-differentiation [25,28]. Furthermore, TNF-α down-regulated anti-inflammatory adiponectin production from human adipocytes [29]; however, leptin production was found to be transiently up-regulated and subsequently down-regulated within 24–48 h [30–32]. Both TNF-α and IL-6 levels have been found to be higher in obese than in lean subjects (in plasma and/or adipocyte tissue [26,27]), but paradoxically the in vivo lipolytic rate in adipocytes from obese individuals apparently does not seem to dominate (i.e. no reduction of adipose tissue mass). The net outcome of this persistent low-grade pro-inflammatory condition in obesity leads to high leptin and to low adiponectin concentrations [26,27]. Interestingly, diet-induced weight loss in obese individuals resulted in diminished TNF-α, IL-6, and leptin concentrations, and in augmented adiponectin expression [27].

The contrasting immunomodulatory effects of adipocyte tissue-derived leptin and adiponectin in conjunction with the apparent cross-talk interaction between, mainly, pro-inflammatory cytokines and adipocyte tissue, prompted us to study the hypothesis as to whether pro- and anti-inflammatory cytokines were able to induce distinctive, possibly opposing, effects on immunostimulatory leptin and immunosuppressive adiponectin release during in vitro human adipogenesis.

2. Materials and methods

2.1. Subjects

Subcutaneous white adipose tissue samples were obtained from healthy 29–52-year-old women (43 ± 10 years, mean ± SD; $n = 4$) undergoing elective liposuction surgery in thighs/hips. The body mass index (BMI) range of the donors was between 19 and 36 kg/m^2 (27 ± 8 kg/m^2, mean ± SD; two lean and two obese donors). All women were otherwise healthy and free of metabolic (e.g. lipid storage disorder or diabetes) or immune diseases. Informed consent was obtained from each participating donor.

2.2. Cell culture

Purified white adipocyte tissue-derived preadipocytes were purchased from Zen-Bio Inc. (Research Triangle Park, NC, USA). Briefly, preadipocytes were isolated from subcutaneous adipose tissue (see above, Section 2.1) by using a collagenase digestion and centrifugation method as previously described [33]. Preadipocyte preparations were devoid of endothelial cells as exemplified by negative staining for vascular endothelial growth factor receptors (data not shown). Preadipocytes were suspended in DMEM/Ham's F12 medium supplemented with 10% fetal calf serum (FCS), 15 mM HEPES and antibiotics ('Preadipocyte Medium' from Zen-Bio), and seeded in 96-well flat-bottomed culture plates (Costar-Corning, Schiphol-Rijk, The Netherlands). After reaching 100% confluence (day 0), preadipocytes were either put on 'Preadipocyte Medium' to retain preadipocyte characteristics, or on 'Differentiation Medium' containing DMEM/Ham's F12 supplemented with 3% FCS, 15 mM HEPES, 1 μM dexamethasone, 100 nM human insulin, 250 μM isobutyl methylxanthine (IBMX), 10 μM proprietary peroxisome proliferator-activated receptor-γ agonist (an indomethacin-derived compound), 33 μM D-biotin, 17 μM potassium–pantothenate, and antibiotics