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IL-17 inhibits adipogenesis in part via C/EBPα, PPARγ and Krüppel-like factors

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ABSTRACT

IL-17 is an inflammatory cytokine associated with anti-microbial host defense and pathogenesis of autoimmune diseases. Obesity is considered to be an inflammatory condition, but how cytokines and fat metabolism are interconnected remains poorly understood. Mesenchymal stem cells can differentiate into adipocytes, which serve as depots for stored fat. Despite the pro-inflammatory properties of IL-17, both IL-17- and IL-17RA-deficient mice are overweight. Consistently, IL-17 suppresses maturation of cells with adipogenic potential. However, the mechanism underlying IL-17-mediated inhibition is not defined. In this study, we addressed this question by evaluating the impact of IL-17 on a variety of transcription factors (TFs) that control adipogenesis, using 3T3-L1 cells to model adipocyte differentiation. Surprisingly, IL-17 does not suppress adipogenesis via C/EBPβ and C/EBPδ, TFs often considered to be central regulators of adipogenesis. Rather, IL-17 suppresses expression of several pro-adipogenic TFs, including PPARγ and C/EBPα. Moreover, we found that IL-17 regulates expression of several members of the Krüppel-like family (KLF). Specifically, IL-17 suppresses KLF15, a pro-adipogenic TF, and enhances expression of KLF2 and KLF3, which are anti-adipogenic. Thus, IL-17 suppresses adipogenesis at least in part through the combined effects of TFs that regulate adipocyte differentiation.

1. Introduction

Interleukin (IL)-17A is the founding member of a unique family of pro-inflammatory cytokines, composed of IL-17A (referred to here as IL-17), IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F [1]. IL-17 and IL-17F are the hallmark cytokines of Th17 cells, a CD4 + T effector population whose discovery in 2005 revolutionized our understanding of T helper cell biology [2]. A number of innate cell types also produce IL-17 and bear marked functional and phenotypic similarity to Th17 cells [3]. IL-17 is centrally involved in tissue inflammation, acting on epithelial, endothelial and mesenchymal cell types to induce expression of pro-inflammatory chemokines and other factors that induce neutrophil chemotaxis, as well as promoting a highly pro-inflammatory state [4]. Consequently, IL-17A−/− or IL-17R−/− mice are highly susceptible to infection, especially to mucosal pathogens [5].

Conversely, IL-17 and other Th17-derived cytokines promote pathology of autoimmune diseases such as rheumatoid arthritis, psoriasis and lupus. Consistent with its role as a pro-inflammatory cytokine, IL-17 signals cooperatively or synergistically with other inflammatory effectors, particularly TNFα but also IFNγ, LTα, BAFF among others [6–9], which may explain its role in driving autoimmunity. Antibodies to TNFα have long been used in the clinic, and IL-17 and its receptor are now in clinical trials to treat autoimmune disease. Therefore, understanding the broader effects of IL-17 and its interactions with other cytokines such as TNFα beyond the immune system has important clinical implications [10].

In that regard, emerging data indicate that IL-17 regulates non-inflammatory activities, including mesenchymal cell differentiation. In particular, IL-17 was shown to enhance differentiation of mesenchymal stem cells (MSCs) into osteoblasts, and conversely to suppress MSC differentiation into adipocytes [11–13]. Consistently, IL-17A−/− and IL-17R−/− mice are overweight, show defects in glucose metabolism, and experience enhanced bone loss during osteoporosis [14,15]. However, little is understood about how IL-17 regulates these events at a mechanistic level.

The process of differentiation from a precursor preadipocyte to a fully mature adipocyte follows a precisely ordered and temporally regulated series of gene expression events (see Fig. 2A). A number of transcription factors (TFs) regulate adipogenesis, including the peroxisome proliferator-activated receptor (PPAR) [16,17] and CCAAT/enhancer-binding protein (C/EBP) family proteins [18,19]. C/EBPδ and C/EBPβ are induced rapidly, followed by expression of C/EBPα and PPARγ. C/EBPα and PPARγ in turn induce

Abbreviations: KLF, Krüppel like factor; IL-, interleukin; GR, glucocorticoid receptor; Egr-2, early growth response gene2 (KROX 20); Srebp1c, sterol regulatory element binding protein 1c; PPARγ, peroxisome proliferator-activated receptor; GRE, glucocorticoid response element; TF, transcription factor; TG, Triglyceride.

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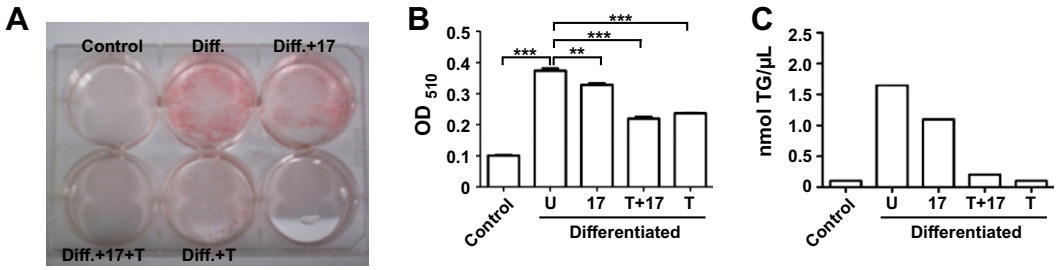

Fig. 1. IL-17 suppresses adipogenesis. (A) 3T3-L1 cells were cultured alone ("control") or in adipogenic culture conditions ("differentiated") in the presence of IL-17 (200 ng/ml) and/or a suboptimal dose of TNFα (2 ng/ml) for 10 days. Cells were stained with Oil Red O to visualize fat formation. (B) Lysates from the cells described in panel A were solubilized and evaluated by light spectroscopy at OD_{510}. (C) Cells were differentiated in duplicate for 10 days with the indicated cytokines and triglyceride content (TG) was assessed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

programs of gene expression leading to the differentiation of mature adipocytes. Additional TFs further influence this process, including sterol-regulated element binding protein 1c (SREBP1c, also known as ADD1, adipocyte differentiation-1), GATA2 and GATA3, and members of the Krüppel-like factor family (KLFs) [16,19,20]. KLF zinc finger proteins play diverse roles in regulation of cell proliferation, differentiation, and development [21,22]. Several members of the KLF family are implicated in adipogenesis. KLF4, KLF5 and KLF15 are pro-adipogenic, while KLF2 and KLF3 exert negative effects on adipogenesis.

Here, we analyzed expression of a panel of TFs that regulate adipogenesis to ascertain where IL-17 might exert its suppressive effects. We show that IL-17 signaling alters expression of several adipogenic TFs in a manner correlating with suppressed adipogenesis, including C/EBPα, PPARγ and several KLF family members, and surprisingly appears to act downstream of C/EBPβ and C/EBPδ. This is the first evidence for a modulatory role of IL-17A on KLF proteins, and provides new insight into the interconnection between IL-17-driven inflammation and adipocyte development.

2. Materials and methods

2.1. Cell culture and differentiation

3T3-L1 cells were from American Type Culture Collection (ATCC) (Manassas, VA). Cells were grown to confluence for 2 days in Dulbecco's modified Eagle's medium (DMEM) (Cellgro, Manassas, VA) supplemented with FCS (GemCell, West Sacramento, CA) Penn-Strep, non-essential amino acids, pantothenate and biotin (Invitrogen, Carlsbad, CA). Differentiation was induced on day 0 with DMEM supplemented with 10% FCS, 0.07 mg/mL insulin (bovine pancreas), 0.4 mg/mL dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 0.003 mg/mL ciglitizone (Sigma, St. Louis, MO) with IL-17A (200 ng/mL) and TNF-α (1–2 ng/mL) (Peprotech, Rocky Hill, NJ). Cells were maintained in DMEM containing 10% FBS after day 4 of post-differentiation.

2.2. Oil Red O staining and TGA assay

Monolayers of differentiated 3T3-L1 cells were washed with PBS and fixed with 10% formalin in PBS for 1 h. After washing with 60% isopropanol, cells were stained with Oil-Red-O (Cayman Chemical, Ann Arbor, MI). Oil-Red-O in isopropanol was diluted with 3/2 volumes of distilled water, filtered and added to the fixed cell monolayers for 1 h at room temperature. Monolayers were washed with distilled water and visualized by microscopy. Quantitation was carried out by extracting Oil-Red-O-stained triglyceride droplets with 100% isopropanol, and OD was measured at 510 nm. Triglyceride content was assessed in 5 µl from duplicate samples 10 days after induction of differentiation with a kit from BioVision (Mountain View, CA), per manufacturer's instructions.

2.3. Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from cells using an RNeasy Mini Kit (Qiagen, Germantown, MD) and reverse transcribed into cDNA using Superscript III Reverse Transcriptase (Invitrogen, Grand Island, NY). Resulting cDNA was then used as a template in quantitative real-time PCR reactions performed using Perfecta SYBR Green FastMix (Quanta Biosciences, Gaithersburg, MD) and various RT^2 qPCR Primer Assays specific for adipogenic genes (SABiosciences, Qiagen) in the ABI-7300 fast detection system (Applied Biosystems, Invitrogen). Relative expression (fold-change vs. undifferentiated control) was quantified by the $2^{-\Delta\Delta Ct}$ method. For normalization, mouse 18s rRNA primers were used. The average value of three separate control sample was set to a value of one and was used to determine fold-change of both controls and experimental groups. Error bars indicate standard deviations.

2.4. Statistical analysis

All values are mean ±s.d. For statistical analysis, the p value was calculated using ANOVA with a post hoc Tukey test, and $p < 0.05$ considered significant. $^{*}p < 0.05$, $^{**}p < 0.005$, $^{***}p < 0.0005$. Ns., not significant.

2.5. Plasmids, transfections and luciferase reporter assays

The amplicons for PCR were generated from 3T3-L1 genomic DNA. The KLF15 promoter was generated by PCR (−765 to −1 relative to the transcriptional start site) using specific primers (sense: 5′-CGCGTAGCAGTGTAGGCTAA-3′; antisense 5′-CTCGAGGCCGGCC CGGCTCCGT-3′) and subcloned into the pGL3-Luc-Enhancer vector (Promega, Madison, WI). The KLF15 enhancer was amplified with the sense primer 5′-CGCGTGCACTGACCCAATGGC-3′ and the antisense primer 5′-GATCCAGGACTTCCTGGACCC-3′ and inserted into the pGL3-Luc-Promoter vector (Promega). For luciferase reporter assays, 3T3-L1 cells were plated into 12-well plates and transfections were performed in triplicate on day 1 after adipogenic induction with or without IL-17, using FuGENE-6 (Roche Applied Science, Indianapolis, IN). After 48 h, cells were lysed and luciferase assays were performed using a Dual-Glo Luciferase kit (Promega).

3. Results

3.1. IL-17A inhibits adipogenesis downstream of C/EBPβ and C/EBPδ and upstream of PPARγ and C/EBPα

We and others previously reported that IL-17 inhibits adipocyte differentiation of adipogenic cell lines and MSC cultures [12,14,15]. The 3T3-L1 cell line can be induced to differentiate into mature adipocytes by a hormonal cocktail that triggers a cascade of