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Leptin modulates autophagy in human CD4$^+$CD25$^-$ conventional T cells

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ABSTRACT

Objective. In this report we show that the adipocytokine leptin directly modulates autophagy in human CD4$^+$CD25$^-$ conventional (Tconv) T cells.

Results. In vitro treatment with recombinant human leptin determined an inhibition of autophagy during T cell receptor (TCR) stimulation, and this phenomenon was dose- and time-dependent. The events were secondary to the activation of the mammalian-target of rapamycin (mTOR)-pathway induced by leptin, as testified by its reversion induced by mTOR inhibition with rapamycin. At molecular level these phenomena associated with Bcl-2 up-regulation and its interaction with Beclin-1, whose complex exerts a negative effect on autophagy.

Materials/methods. The impact of leptin on autophagy of Tconv cells was determined at biochemical level by western blotting and by flow cytometry; the interaction between BCL-2 and Beclin-1 by co-immunoprecipitation assays.

Conclusions. Our results, suggest that in unconditioned, freshly-isolated human Tconv cells, autophagy and proliferation are controlled by leptin during TCR-engagement, and that both phenomena occur alternatively indicating a balance between these processes during immune activation.

1. Introduction

Leptin is a 16 kD adipocyte-derived cytokine that regulates neuroendocrine functions and controls food intake, energy expenditure, glucose and fat metabolisms [1]. It mediates its functions through the long form of the leptin receptor (LepRb) which is part of the class I cytokine receptors, expressed at different levels also on innate and adaptive immune cells

Abbreviations: mTOR, mammalian-target of rapamycin; TCR, T cell receptor; hLepRb, human leptin receptor-b; LC3-II, microtubule associated protein light chain 3-II; Tconv, T conventional.

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including monocytes, dendritic cells (DCs), B and T cells, respectively [2]. In this context, it has been previously suggested that leptin represents an important link among nutritional status, metabolism, and immune responses [3,4], indeed it signals to $CD4^+$ T cells that sufficient amount of energy is stored as fat to support the increased energy demand during immune responses against pathogens [5]. Also, leptin shows differential effects on several T cell subpopulations [6,7] through the activation of the mammalian target of rapamycin (mTOR) pathway [7–9]. mTOR is a molecular sensor of cellular nutritional status and integrates signals from the environment to the nucleus for the regulation of cell metabolism, proliferation, survival and autophagy [10]. mTOR plays a negative role on cellular autophagy [11–14]: autophagy, from the Greek words, *auto* "self" and *phagein* "to eat", is the basic catabolic mechanism that involves cell degradation of unnecessary or dysfunctional cellular components through the actions of lysosomes. The breakdown of cellular components can ensure cell survival during reduced energy availability (ie. starvation) by maintaining cellular energy levels [15]. During this process, targeted cytoplasmic constituents are isolated from the rest of the cell within the autophagosomes, which are then fused with lysosomes and degraded or recycled. The molecular mechanism by which mTOR inhibits autophagy is not completely understood. Over the past few years, autophagy has been considered as a process that provides a survival advantage to cells undergoing nutrient deprivation or other stresses [13–15]. Indeed genetic or pharmacological alterations in autophagy impair cell survival rate or cell metabolism, thereby affecting tissue homeostasis. In the context of the immune system, recent papers have shown that autophagy may be also linked to apoptosis [16–18] and might play different roles in lymphocyte development [19–21] and function, by maintaining the normal number of B, $CD4^+$, $CD8^+$ T cells [22,23] and controlling T cells activation [18], thymic selection [24] and antigen presentation [25]. These data indicate that autophagy plays a role in switching the cell fate toward differentiation or specific functional commitments, such as T cell polarization, suggesting that metabolic state (through leptin) might influence this process. Moreover, leptin has been demonstrated to exert opposite effects on human regulatory $CD4^+CD25^+$ (Treg) and conventional $CD4^+CD25^-$ (Tconv) T cells: indeed it inhibits Treg cell proliferation [6], on the one side, whereas it enhances Tconv proliferation, on the other [7]. These effects on both cellular subsets were induced by mTOR activation [7,8]. Particularly, on Tconv cells the enhancement in their proliferation associated with inflammatory cytokine secretion, whereas leptin neutralization determined the inhibition of their responses, thus suggesting a key role of this adipocytokine in Tconv cells homeostasis and function and in pathogenesis of several inflammatory and autoimmune disease [26]. Levels of leptin are, in fact, typically low during infection and high in autoimmune disorders, both systemically and at the site of inflammation (ie. multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA)) [26,27]. In this context, this report investigates the role of leptin, considered as a "dominant" peripheral signal of fuel availability, on the modulation of the autophagic process in the context of human Tconv cells biology.

2. Materials and methods

2.1. Cell cultures, purification and proliferation assays

The leptin-dependent BAF/3-$LepRb^+$ cell line, stably transfected with the long form of human leptin receptor was kindly provided by Prof. Arieh Gertler from the Hebrew University, Rehovot, Israel. In brief, BAF/3-$hLepRb^+$ cells were cultured in RPMI-1640 medium in the presence of human leptin (10 ng/mL), supplemented with 10% FCS, 2 mmol/L L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin (Life Technologies, Carlsbad, CA). Cells were cultured at 37 °C in 100% humidity and 5% CO_2. To evaluate the leptin effects on autophagy, in all experiments, BAF/3-$hLepRb^+$ cells (1–2 × 10^6 cells/well) were leptin deprived for 12 h and left in low serum (2% FCS). The day after they were acutely stimulated with recombinant human leptin (100 ng/mL) (R&D Systems, Minneapolis, MN) at times shown in figures.

Human $CD4^+CD25^-$ Tconv cells were purified from PBMCs from buffy coats of human healthy donors by either magnetic cell separation with the Dynabeads Treg Cell Kit (Invitrogen) or by FACS sorting (MoFlo, Dako-Beckman-Coulter), and Tconv cells purity was always between 95 and 98%. The study was approved by the Federico II Ethics Review Board and donors gave informed written consent for the blood donation. Cells (1–2 × 10^6 cells/well) were washed extensively with serum free culture medium, left in low serum [2% AB human serum (Invitrogen)] for 2 h at 37 °C in presence of lysosomal protease inhibitor [NH_4Cl (20 mmol/L) and leupeptin (100 μmol/L) (Sigma)], and after were washed and stimulated or not with recombinant human leptin (100–200 ng/mL) (R&D Systems, Minneapolis, MN) plus anti-CD3/CD28 mAbs coated beads (0.2 beads/cell, Invitrogen) for 2 h at 37 °C. For proliferation assays, Tconv cells were stimulated or not with human leptin (200 ng/mL) and human leptin-neutralizing mAb (R&D Systems, Minneapolis, MN), used at a final concentration of 0.25 to 25 mg/mL. For transient mTOR inhibition, either BAF/3-$hLepRb^+$ or Tconv cells were pre-treated for 1 h with rapamycin (Sigma-Aldrich) at final concentration of 100 nmol/L. Cells were stimulated for 3 days, labeled with [3H]thymidine (0.5 mCi/well) (Amersham-Pharmacia Biotech, Cologno Monzese, Italy) for the last 16 h of culture, and harvested after 12 h (Tomtec). Radioactivity was measured with a β-cell-plate scintillation counter (Wallac, Gaithersburg, MD). For mouse studies, 8–10-wk-old C57BL/6J (B6) wild-type (WT), and leptin–deficient mice (C57BL/6J-*ob/ob*) were purchased from Charles River Laboratories (Calco, Italy). The study was approved by the Ethical Veterinary Board of the Federico II University.

For biochemical analyses on mouse cells, 1–2 × 10^6 Tconv cells (isolated by negative selection using the Miltenyi Biotec Treg Cell Isolation kit and an AutoMACS cell separator, cell purity >95%), were obtained from the splenocytes of each group of WT or C57BL/6J-*ob/ob* mice and left for 12 h in 2% FCS, 2 mmol/L L-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin (Life Technologies, Carlsbad, CA). The day after, cells were stimulated for 2 h with mouse recombinant leptin (200 ng/mL) (R&D Systems, Minneapolis, MN).