

Leptin: molecular mechanisms, systemic pro-inflammatory effects, and clinical implications

Leptina: mecanismos moleculares, efeitos pró-inflamatórios sistêmicos e implicações clínicas

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SUMMARY

Leptin, the adipokine produced mainly by the white adipose tissue, plays important roles not only in the regulation of food intake, but also in controlling immunity and inflammation. It has been widely demonstrated that the absence of leptin leads to immune defects in animal and human models, ultimately increasing mortality. Leptin also regulates inflammation by means of actions on its receptor, that is widely spread across different immune cell populations. The molecular mechanisms by which leptin determines its biological actions have also been recently elucidated, and three intracellular pathways have been implicated in leptin actions: JAK-STAT, PI3K, and ERK 1/2. These pathways are closely regulated by intracellular proteins that decrease leptin biological activity. In this review, we discuss the molecular mechanisms by which leptin regulates immunity and inflammation, and associate those mechanisms with chronic inflammatory disorders. *Arq Bras Endocrinol Metab.* 2012;56(9):597-607

Keywords

Leptin; immunity; inflammation; cytokines

SUMÁRIO

A leptina, uma adipocina produzida principalmente pelo tecido adiposo branco, tem um papel importante não somente na regulação da ingestão alimentar, mas também no controle da imunidade e da inflamação. Já foi amplamente demonstrado que a ausência de leptina causa deficiências imunológicas em modelos animais e em humanos, levando ao aumento da mortalidade. A leptina também regula a inflamação por meio da ação em seu receptor, amplamente distribuído em diversos tipos de células do sistema imunológico. Os mecanismos moleculares pelos quais a leptina determina suas ações biológicas foram recentemente elucidados, e três cascatas intracelulares são ativadas pela leptina: JAK-STAT, PI3K e ERK 1/2. Essas cascatas são reguladas por proteínas intracelulares, reduzindo as ações da leptina. Nesta revisão, são discutidos os mecanismos moleculares pelos quais a leptina regula a imunidade e a inflamação, associando-os a enfermidades inflamatórias crônicas. *Arq Bras Endocrinol Metab.* 2012;56(9):597-607

Descritores

Leptina; imunidade; inflamação; citocinas

INTRODUCTION

Although initially considered as a simple storage of fat, overwhelming evidence led the adipose tissue to be conceptualized as an endocrine organ (1,2). The discovery of leptin has dramatically changed the understanding of the physiological importance of the adipose

tissue, making it clear that the adipose tissue synthesizes and releases this key hormone that plays a crucial role to signal the brain to control food intake and energy expenditure (3). From this seminal discovery to the present, it has been shown that the adipose tissue not only synthesizes and releases dozens of other metabo-

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Received on May/27/2012
Accepted on Oct/24/2012

lic-related factors, such as resistin and adiponectin, but it is also a source of cytokines and immune factors such as tumor-necrosis factor alpha (TNF- α), interleukin-1 (IL-1) alpha (IL-1 α), IL-1 β , IL-1 receptor antagonist (IL-1RA), and IL-6 (1). All of these findings attracted attention to fat as an immunoendocrine organ that interacts with several physiological systems of the organism.

Leptin is a cytokine produced predominantly by the differentiated white adipose tissue, with various functions in the immune and endocrine systems, including reproduction, glucose homeostasis, hematopoiesis, angiogenesis, osteogenesis, wound healing, and inflammation (4). This hormone regulates caloric expenditure and intake, playing a central action in energy balance (5). Leptin levels are directly correlated with fat mass and are elevated in obese patients, who are also leptin-resistant. Leptin-deficient mice and humans are severely obese and have several metabolic and endocrine alterations, such as hyperglycemia, insulin resistance, hypertriglyceridemia, hypogonadotropic hypogonadism, and central hypothyroidism (6,7).

Leptin plays a major role in the chronic pro-inflammatory state that is seen in obesity, metabolic syndrome, and their complications, such as atherosclerosis (8). By acting on the long isoform of the leptin receptor (Ob-R), which is expressed by different immune cell types, leptin triggers inflammatory responses. Concomitantly, certain inflammatory and infectious stimuli, such as IL-1, lipopolysaccharide (LPS), and TNF- α can also increase leptin levels (9), which correlate with the level of inflammation. Therefore, the interactions between leptin and inflammation are bidirectional: pro-inflammatory cytokines increase the synthesis and release of leptin, which in turn helps to perpetuate the loop of chronic inflammation in obesity.

In this manuscript, we summarize the role of leptin in the immune system, the molecular links between leptin and inflammation, and the relationships between leptin and chronic inflammatory diseases.

Molecular mechanisms of leptin and modulation of immune cells

During states of normal nutrition, leptin signals to the brain that excess energy is available for several biological processes, including immune response. Under energetic restriction, leptin levels are low, and immune response is impaired. Leptin exerts a link between the T helper (Th) 1 immune response and nutritional status, and inadequate body weight – both obesity and malnutri-

tion – can importantly influence immune cell function, both in innate and adaptive immune responses (10).

Leptin has structural homology with the cytokines of the long-chain helical family that includes IL-6, IL-11, IL-12, and oncostatin M. Leptin receptor, Ob-R, has structural similarities with the members of the class I cytokine receptor (gp130) superfamily, which includes the receptor for IL-6, leukocyte inhibitory factor (LIF), and granulocyte colony-stimulating factor (G-CSF). The Ob-R is expressed by neutrophils, monocytes, macrophages, subpopulations of T cells and B cells, mast cells, dendritic cells (DC), and natural killer (NK) cells (11). There are at least six different isoforms of the leptin receptor in rodents; Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, Ob-Re, and Ob-Rf; these are all products of six alternatively spliced forms of the Ob-R gene (12). Of all of these isoforms, Ob-Rb is the only long form, which contains a prominent cytoplasmic region containing several motifs required for signal transduction and is capable of activating the JAK-STAT pathway. There are four truncated (short) forms, including Ob-Ra, Ob-Rc, Ob-Rd, and Ob-Rf, of which Ob-Ra is regarded as a leptin transporter across the blood-brain barrier and a leptin degrader; and a secreted form (Ob-Re) that lacks both the intracellular and transmembrane domains, and serves as a plasmatic leptin-binding protein. These isoforms are involved in mediating leptin actions in the brain and peripheral organs. All isoforms, with the exception of Ob-Rb, share the same identical extracellular, ligand-binding domains, but differ at the C terminus. Only Ob-Rb has a long cytoplasmic region containing several motifs required for signal transduction, whereas the others have transmembrane domains, and lack some or all of these motifs. Transduction signaling takes place by activation of conserved box 1 and box 2 motifs (intracellular amino acids 6-17 and 49-60, respectively). Cytokine receptor homology module 2 (CRH 2) is the main binding site for leptin on the Ob-R. The immunoglobulin-like domain (Ig-like) and fibronectin III domain (FNIII) are critically involved in Ob-R activation. The role of CRH1 remains to be determined.

The elucidation of leptin-induced second messenger pathways is marked by two fundamental factors: 1) leptin tridimensional structure and 2) leptin resistance to perform as an anti-obesity hormone in obese/overweight non-leptin deficient individuals. First, although the cloning of the *Ob* gene revealed no significant sequence homology with any other known protein, com-

putational-based research studies predicted that leptin displayed tridimensional characteristics of a four-helix bundled cytokine, such as those that activate the JAK-STAT pathway (Figure 1) (13). Moreover, the cloning of the *OB-R* gene reported towards the end of 1995 (12) revealed that leptin receptor shared sequence homology with gp130, the signal transducing unit of the IL-6 receptor that signals via the JAK-STAT pathway. The structural similarities that leptin and the *OB-R* gene share with the IL-6 cytokine family lead to functional *in vitro* signaling studies that proved that leptin activated the JAK-STAT pathway (14). *In vivo* studies carried out in mice have shown that peripheral administration of leptin-activated hypothalamic STAT-3 in *ob/ob* and WT mice, but not in *db/db* mice, indicating that the presence of a functional *OB-R* gene was required for leptin-induced STAT-3 activation within the brain.

Leptin controls other two key signaling pathways, namely the extracellular signal-regulated kinase (ERK)-1/2 (Figure 2), and the activation of the phosphatidylinositol-3-kinase (PI3K) (Figure 3) (15). Janus-activated kinase phosphorylation of Tyr⁹⁸⁵ (or phosphorylated JAK2 itself, independent of phosphor-

ylated Tyr⁹⁸⁵) leads to recruitment and phosphorylation of SHP-2 (*src* homology 2-containing tyrosine phosphatase). Then, phosphorylated SHP-2 activates the ERK signaling pathway via growth factor receptor binding-2 (GRB-2) (16). The ERK/MAPK pathway is believed to be the main mechanism involved in regulatory T (T_{reg}) cell regulation (11), by controlling the promoters for the transcription of *c-fos*, *jun* and *egr-1* genes, fundamental in cell proliferation and differentiation (17). The ERK/MAPK pathway can be activated by both Ob-Ra and Ob-Rb leptin receptors. It shares many similarities with the JAK2/STAT3 pathway as JAK2 is required for phosphorylation of the SH2-domain containing protein tyrosine phosphatase (SHP-2) via pathways both dependent and independent of Tyr⁹⁸⁵ (18).

There is also one pathway that occurs independently of phosphorylation at tyrosine sites on Ob-Rb, leading to the activation of phosphatidylinositol 3'-kinase (PI3K). This pathway originates with JAK2 autophosphorylation, which leads to the recruitment and phosphorylation of insulin receptor substrate (IRS) proteins. Then, phosphorylated IRS proteins recruit phosphatidylinositol 3'-kinase (PI3K) and activate downstream signals (15).

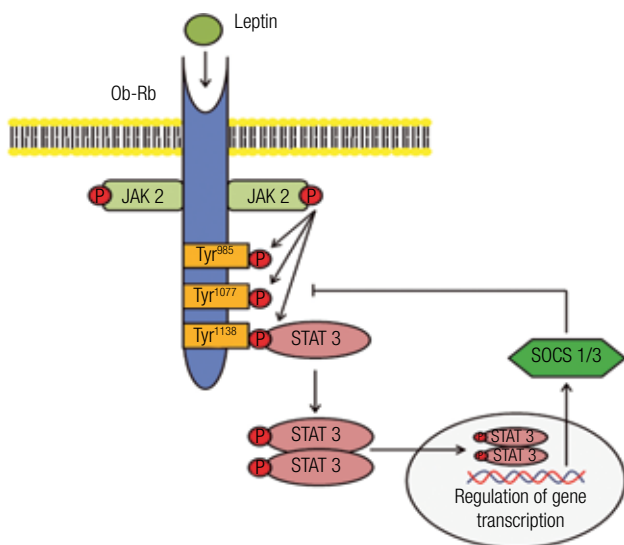


Figure 1. Leptin and the JAK-STAT pathway.

Leptin binds to the leptin receptor (Ob-Rb) and activates Janus-family tyrosine kinase 2 (JAK2) by auto- or cross-phosphorylation. JAK2 then phosphorylates the other tyrosine residues (Tyr⁹⁸⁵, Tyr¹⁰⁷⁷, Tyr¹¹³⁸), which act as sites for the binding of intracellular signaling molecules. Signal Transducer and Activator of Transcription 3 (STAT-3) is a substrate of JAK 2 and subsequently binds to the phosphorylated Tyr¹¹³⁸. STAT-3 then undergoes dimerization and translocates into the cell nucleus to function as a promoter for gene transcription [4]. Members of the SOCS family (SOCS1 and SOCS3) suppress the action of leptin by binding to JAK 2 and tyrosine residues.

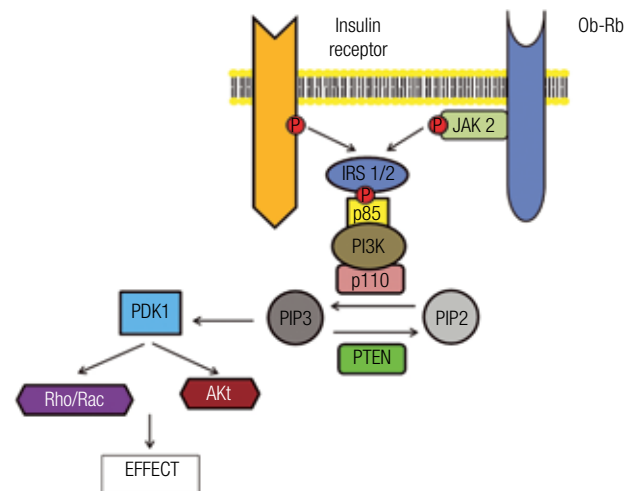


Figure 2. Leptin and the PI3K pathway.

Both the leptin and insulin receptors play a role in the initial step of IRS 1/2 phosphorylation, which then binds to the regulatory p85 subunit of PI3K. The active p110 subunit then adds a phosphate to the 3' position of PtdIns 4,5-diphosphate (PIP2) forming PtdIns 3,4,5-triphosphate (PIP3), which regulates proteins that contain the pleckstrin homology (PH) domain. These include the isoforms of protein kinase C, phosphoinositide-dependent kinase 1 (PDK1), and protein kinase B (Akt), which is activated by PDK1.

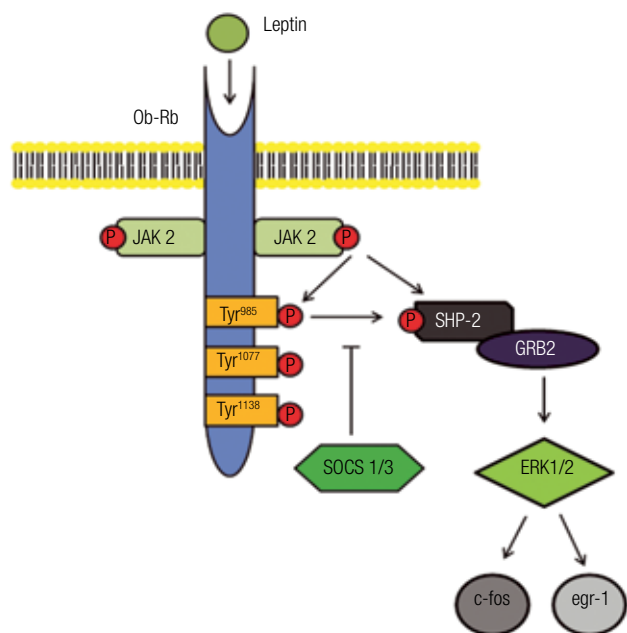


Figure 3. Leptin and the ERK pathway.

Stimulation of Ob-Rb promotes phosphorylation of Tyr985 by JAK2. Both these units can phosphorylate SHP-2, which further modulates GRB2 and activates ERK1/2 of the MAPK family. ERK1/2 promotes the transcription of the *c-fos* and *egr-1* genes. SOCS3 is an inhibitor of this pathway, with a mechanism similar to the JAK2/STAT3 pathway.

The PI3K pathway is an acute phase contributor of inflammation, in contrast to JAK2/STAT3, which is a slower process as it functions by altering gene transcription (15). Both JAK2 and the insulin receptor participate in the phosphorylation of IRS, which then stimulate the p85 subunit of PI3K, thereby activating it. Once activated, the main mechanism of action of PI3K is intracellular phosphorylation of proteins. The chain of events involve phosphorylation of PtdIns 4,5-diphosphate (PIP2) to form PtdIns 3,4,5-triphosphate (PIP3) (19). Phosphatase and tensin homologue (PTEN) is an antagonist of this pathway and fosters the conversion of PIP3 back to PIP2 (Figure 3) (19). PIP3 is a key activator of phosphoinositide-dependent kinase 1 (PDK1), which has many downstream effects, especially in regulating protein kinase B (Akt). Akt is a key participant in regulating neuropeptide Y and nitric oxide (NO) mTOR via p70S6K (19). The PI3K pathway has also been implicated in controlling the Rho family of GTPases, which regulate apoptosis and remodel the actin skeleton within cells (15,19).

Soon after leptin discovery, it became evident in pre-clinical and clinical experiments that obese rodents and

humans displayed leptin resistance. Thus, the search for the putative mechanisms leading to leptin resistance was taking place. In the mid-90s, a new family of cytokine-induced intracellular signaling inhibitors of the JAK-STAT pathway were identified, namely the suppressors of cytokine signaling (SOCS 1-3) (20). SOCS3 plays a key role in providing negative feedback to this pathway, as it binds to Tyr⁹⁸⁵ and JAK2 to prevent phosphorylation of SHP-2, thus stopping the cascade. It is also believed to repress the activity of SHP-2 (21). Since leptin also operates via JAK-STAT, Bjorbaek and cols. hypothesized that activation of SOCS genes could contribute to leptin resistance (22). They demonstrated that peripherally administered leptin rapidly increased the induction of hypothalamic SOCS3 in areas that are relevant for the control of food intake and energy expenditure. Moreover, they showed that SOCS3 was involved in negative regulation of leptin-induced intracellular signal transduction. In rodent models, it was shown that SOCS3 could also be involved in leptin resistance that occurs during the aging process, and that lack of SOCS3 within the brain was effective to increase leptin sensitivity and to prevent diet-induced obesity (23).

Another intracellular mechanism that was shown to control leptin resistance is orchestrated by protein tyrosine phosphatase 1B (PTP1B) (24). This phosphatase acts by dephosphorylating JAK2 and, therefore, it prevents JAK2-induced phosphorylation of the leptin receptor (24). Moreover, similar to mice deficient in SOCS3, neuronal deletion of PTP1B increased leptin and insulin sensitivity, preventing body weight gain in a diet-induced obesity animal model (25). Similar findings were previously shown in whole-body PTP1B knock-out studies (26).

Leptin and innate immunity

Leptin binds to its receptor in macrophages and monocytes, improving phagocytosis by regulating oxidative stress. It also induces eicosanoid and nitric oxide synthesis, acts as a chemoattractant, increases the secretion of cytokines, such as IL-1RA, IL-1, IL-6, TNF- α , and CC-chemokine ligand, and prevents apoptosis (27). It also dose-dependently increases the proliferation of circulating cells, and stimulates the expression of activation markers, such as CD69 and CD25, among others (28). The increased number, and the activation of monocytes by the phorbol-12 myristate 13-acetate (PMA) or LPS is synergistically improved by leptin (29). Lep-

tin also activates macrophages by means of the mammalian Target of Rapamycin (mTOR) kinase pathway, which is an intracellular nutrient-response-dependent pathway that integrates growth factor and nutrient-derived signals to cellular growth rates, controlling cell growth and division. Leptin stimulates phagocytosis by stimulating phospholipase and increasing the production of leukotriene B₄, eicosanoids, NO, cholesterol acyltransferases-1, and cyclooxygenase 2 (30). Leptin also increases the production of growth hormone by means of PKC and NO-dependent pathways (31).

Leptin acts on several other immune cells. On eosinophils, it induces the expression of adhesion molecules and CD18, increases chemokinesis, and stimulates the release of inflammatory cytokines IL-1 β , IL-6, IL-8, growth-related oncogene- α , and monocyte chemoattractant protein-1 (MCP1), which is a known chemoattractant for monocyte/macrophage infiltration (32). On DC, leptin increases the expression of cytokines, such as IL-6 and TNF- α ; surface molecules, such as CD1a and CD80; and reduces apoptosis rates. Leptin also induces morphological and functional changes within DC, directing them towards Th1 priming (33). Mast cells also express Ob-R, and it is probable that leptin acts on them both in a paracrine and/or autocrine fashion (34). On polymorphonuclear cells, leptin induces chemoattraction and the production of reactive oxygen species (ROS) via mechanisms that might involve interaction with monocytes (35). Finally, leptin contributes to NK cell development, differentiation, activation, proliferation, and cytotoxicity (35).

Leptin and adaptive immunity

Leptin maintains the thymic parenchyma via its direct anti-apoptotic effects on T cells by stimulating the medullary thymic epithelial cell expression of IL-7, a thymocyte growth factor (36). Moreover, leptin affects the activation of T lymphocytes. Hypoleptinemia caused by starvation leads to thymic atrophy and higher frequency of infections, and exogenous recombinant leptin prevents the reduction in cortical double-positive CD4⁺CD8⁺ thymocyte subpopulation (37). However, leptin can only induce the proliferation and activation of mature human peripheral blood lymphocytes if it is co-administered with other general immunostimulants, such as concanavalin A (ConA) or phytohemagglutinin (PHA) (36). Importantly, the effect of leptin on lymphocyte proliferation is specific to different subpopulations. It inhibits the proliferation of memory T cells

(CD4⁺CD45RO⁺), but stimulates the proliferation of naive T cells (CD4⁺CD45RA⁺). This means that leptin polarizes Th0 cytokine production towards a pro-inflammatory (Th1, TNF- α , IFN- γ , IL2, IL12, and leptin itself), rather than anti-inflammatory phenotype (Th2, IL-4, IL-10) (38). It seems that leptin acts directly on circulating T lymphocytes when they are co-stimulated, since this effect is observed even in the lack of monocytes (38). On the subpopulation of Th1 cells, leptin promotes IgG2a switching in B cells and induces TNF- α and IFN- γ production, while it exerts inhibitory actions on Th2 cells and IgG1 switching (39). Additionally, leptin is also fundamental for Th2 cell development. For example, in an animal model of Th2-dependent intestinal inflammation, leptin deficiency was associated with decreased Th1 and Th2 polarization, and *ob/ob* mice were protected from intestinal inflammation. Indeed, after polarizing *ob/ob* naive and wild-type (WT) T cells in the Th2 direction, there was suppression of T-cell polarization in the cells of leptin-deficient mice (27).

Furthermore, leptin is able to reduce the human CD4⁺CD25⁺ T_{reg} cells, which are a small subset of CD4⁺ T cells that control the peripheral immune tolerance, and prevent inappropriate immune responses, such as allergy and autoimmunity. Commonly, T_{reg} cells influence the activities of cells of the innate immune system and modulate effector T cell responses. In animal models of chronic leptin and Ob-R deficiencies, the percentage, absolute number, and activity of T_{reg} cells is increased, resulting in resistance to autoimmune diseases. When leptin is replaced, the number of T_{reg} cells returns to the same levels found in WT mice. In humans, leptin has a similar effect on T_{reg} cells. Interestingly, freshly isolated T_{reg} cells can produce significant quantities of leptin, and express high density of the Ob-R, which constrain their proliferation (28).

Besides the effects on CD4⁺ T cells, leptin administration can stimulate the proliferation of B lymphocytes, NKT and CD8⁺ T cells, and increase cytokine responsiveness. The interactions between leptin and cytokines are bidirectional, and both stimulate each other (Figure 3). Table 1 summarizes the leptin effects on innate and adaptive immunity.

LEPTIN AND CLINICAL CONDITIONS

Congenital deficiency of leptin

In humans, congenital leptin deficiency (40) or leptin-receptor mutation (41) lead to increased vulnerability

to infections with elevated risk of death during childhood. Congenital deficiency of leptin is associated with a decrease in CD4⁺ T cell numbers, and an increase in CD8⁺ T and B cells, as well as a pronounced reduction in the number of naive T cells, and an increase in memory T cells. In addition, lymphocytes exhibit impaired proliferative response and reduced production of cytokines in response to several stimuli, with abolishment of IFN- γ secretion, and a prevailing Th2 cytokine phenotype (40). However, these immune defects are not an obligatory characteristic of patients with mutation in the leptin gene, in whom the immune profile possibly depends on the genotype and genetic expressivity (42). In leptin-deficient children, leptin treatment increased proliferation and cytokine response, particularly of IFN- γ levels, increased number of leukocytes, and improved recurrent dermatitis and asthmatic crises (43).

Table 1. Leptin effects on innate and adaptive immunity

	Innate		Adaptive
Monocytes/ macrophages	<ul style="list-style-type: none"> ↑ Phagocytosis ↑ Proliferation ↑ IL1, IL6, TNF 	Thymocytes	<ul style="list-style-type: none"> ↑ Maturation ↓ Apoptosis
Monocytes	<ul style="list-style-type: none"> ↑ Expression of activation markers ↑ Expression of surface markers 	Naive T cells	<ul style="list-style-type: none"> ↑ Proliferation ↓ Apoptosis
Macrophages	<ul style="list-style-type: none"> ↑ Eicosanoids, NO, LB4, CTA1, COX-2 	Memory T cells	<ul style="list-style-type: none"> ↓ Proliferation
Neutrophils	<ul style="list-style-type: none"> ↑ Formation and release of ROS ↑ Chemotaxis ↓ Apoptosis ↑ Expression of CD11b 	Th1 cell activation	<ul style="list-style-type: none"> ↑ IgG2a switch ↑ TNF-α, ↑ INF-γ
Eosinophils	<ul style="list-style-type: none"> ↑ Adhesion molecules ↑ Chemokinesis ↑ Release of IL-1β, IL-6, IL-8 	Th2 cell inhibition	<ul style="list-style-type: none"> ↓ IgG1 switch
Dendritic cells	<ul style="list-style-type: none"> ↑ IL-1β, IL-6, IL-8, IL-12, TNF-α ↑ Maturation ↓ Apoptosis 	T _{reg} cell	<ul style="list-style-type: none"> ↑ Anergy ↑ Hyporesponsiveness
Natural killer	<ul style="list-style-type: none"> ↑ Proliferation, maturation ↑ Differentiation, activation, cytotoxicity 	B cells NKT cells	<ul style="list-style-type: none"> ↓ Apoptosis ↑ Proliferation

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IL: interleukin; NO: nitric oxide; LB4: leukotriene B4; CTA1: cholesterol acyltransferase 1; COX-2: cyclooxygenase-2; TNF: tumor necrosis factor; ROS: reactive oxygen species; INF: interferon.

Patients with leptin-receptor mutation present less prominent immune alterations than patients with congenital leptin deficiency (41). A reduction in the number and function of CD4⁺ T cells, a decrease in cytokine production after antigenic charge, and a compensatory increase in the number of B cells have been described. These alterations are also restored with leptin replacement (41).

Hypoleptinemic states

Malnutrition or caloric deprivation produces immunosuppression in humans and animals. Acute hypoleptinemia caused by starvation is associated with reduced development of B cells in the bone marrow, and decreased T-lymphocyte subpopulation with impairment of delayed-type hypersensitivity responses and thymic atrophy. These effects are more pronounced in patient submitted to chronic exposure to hypoleptinemia, and are prevented by replacement with exogenous leptin (44).

Women with hypothalamic amenorrhea due to chronic caloric restriction (e.g., strenuous exercise or low body weight) present hypoleptinemia and low levels of soluble TNF- α receptor that are normalized by the treatment with r-metHuLeptin (recombinant methionyl human leptin) (45).

Congenital or acquired severe lipodystrophy is characterized by an almost complete lack of adipose tissue and low leptin levels. In general, these patients exhibit measurable abnormalities in immune markers at baseline, such as a higher percentage of B cells and reduction in TNF- α production by peripheral blood mononuclear cells. Leptin replacement can increase CD4, CD8T, and NKT cell numbers, correct the disproportion of B cells, and improve TNF- α release to physiological levels. However, patients with severe lipodystrophy surprisingly do not show clinical immune deficiency, and may present autoimmune diseases including autoimmune hepatitis, thyroidopathies, nephropathies, and type 1 diabetes (46).

Hyperleptinemic states: obesity and metabolic syndrome

Obesity, especially visceral adiposity, is considered an inflammatory disease, since the adipose tissue is able to produce inflammatory cytokines or collaborate with their production by other tissues (2). Also, obese patients exhibit higher level of inflammatory markers such as C-reactive protein, TNF- α , IL-6, IL-18, MIF (macrophage migration inhibitory factor), haptoglobin, SAA (serum amyloid A), and plasminogen activator inhibitor-1. Furthermore, type 2 diabetes, athe-

rosclerosis and other diseases related to obesity are causally linked to inflammation.

Hyperleptinemia is correlated with pro-inflammatory responses and with the chronic sub-inflammatory state observed in obesity. On the one hand, leptin enhances the production of inflammatory cytokines, and on the other hand, cytokines such as IL-6 and TNF- α promote leptin production by the adipose tissue as well. Increased leptin resistance associated with high levels of free fatty acid and inflammatory cytokines may contribute to the reduction in lipid oxidation in insulin-sensitive organs, leading to accumulation of lipids (lipotoxicity) and insulin resistance (47).

Moreover, leptin induces cholesterol uptake by macrophages, angiogenesis, platelet aggregation, stimulates the oxidative stress in endothelial cells and inhibits vasorelaxation, increasing the risk of atherosclerosis (48). Indeed, *ob/ob* mice are resistant to atherosclerosis despite all the metabolic risk factors, whereas apolipoprotein E-deficient mice develop atherosclerosis if treated with leptin (49).

In humans, leptin is an independent risk factor for coronary artery disease, and its levels are correlated with C-reactive protein, plasma triglycerides, and fasting plasma glucose levels (50). Leptin is associated with insulin resistance and metabolic syndrome independent of obesity via central obesity, possibly by impairment of insulin signaling at the insulin receptor substrate-1 phosphorylation (51). Leptin is increased in patients with metabolic syndrome, but relative hypoleptinemia may be observed in cases of severe metabolic syndrome, possibly due to adipose tissue dysfunction (52).

Leptin and acute inflammation

Leptin regulates the production of pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF- α), interleukin 1 (IL-1), and interleukin (IL-6). These cytokines also regulate the expression of leptin, which sustains a chronic pro-inflammatory state (Figure 4).

Many genes related to inflammation, including genes encoding acute phase response proteins such as tPA (tissue plasminogen activator), fibrinogen- β , lipocalin-2, PAPI, preprotachykinin, and MnSOD (manganese superoxide dismutase) are induced by leptin (53). Leptin levels rapidly increase in acute inflammatory conditions, such as cholecystectomy, acute infection, and sepsis, particularly favored by cytokines, such as TNF- α , IL-6, and IL-1 β (36). Parenteral administration of LPS, commonly used to experimentally induce systemic inflammation, leads to a rise in plasma leptin.

A protective role of leptin in the clearance of pathogens is observed in leptin-deficient *ob/ob* mice, which develop severe disease and die of infection with *Klebsiella* more rapidly than WT mice. The *ob/ob* mice are also highly susceptible to LPS-induced lethality, which can be reversed by the administration of leptin. The protective effects of leptin in these cases seem to occur by means of a modulation of TNF- α and IL-6 responses after endotoxin priming (10).

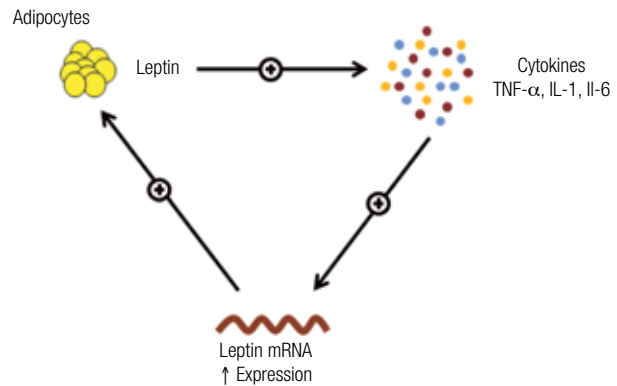


Figure 4. Interaction between leptin and cytokines.

Leptin upregulates the production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF- α), interleukin 1 (IL-1), and interleukin (IL-6). These cytokines increase the expression of leptin mRNA, which subsequently sustains production of leptin. This is thought to be the mechanism for chronic inflammation.

Elevated levels of leptin are found in sepsis and may be predictive of the severity of sepsis and increased survival (54). Leptin is a critical factor in host resistance and in its absence, sepsis-induced organ damage is increased, whereas neutrophil function is diminished. Furthermore, there is an important role of leptin in the central nervous system (CNS) in regulating survival and systemic immune response in sepsis. Selective leptin administration into the CNS controls systemic immune response in a functionally relevant manner with significant protection from sepsis. A leptin-dependent neurocircuit in the CNS is required for efficient coordination of the immune response in sepsis to limit organ damage and prevent mortality (38). On the other hand, some studies have not found increased leptin levels in specific inflammatory conditions, including acute experimental endotoxemia in humans, HIV infection, and newborn sepsis (30).

Leptin in chronic inflammatory diseases

Together with the established function of leptin as a pro-inflammatory cytokine that helps the host fight infection, there is increasing evidence of an association between lep-

tin and increased risk of both chronic inflammatory and autoimmune disease. Serum leptin levels are elevated in many chronic inflammatory conditions (Table 2). In experimental animal models, the investigation of the effects of leptin on the susceptibility to autoimmunity has clearly indicated that leptin can promote autoreactivity. Additionally, leptin-deficient *ob/ob* mice and leptin receptor-deficient *db/db* mice are resistant to the development of several experimentally-induced autoimmune diseases.

Table 2. Leptin and inflammatory diseases

Disease	Effects of leptin
Obesity	Pro-inflammation and atherosclerosis
Type 2 diabetes	Pro-inflammation and atherosclerosis
Sepsis	Higher levels associated with survival
Multiple sclerosis	Activation of myelin-reactive T cells
Inflammatory bowel disease	Pro-inflammation and anorexia
Osteoarthritis	Increases matrix metalloproteinases and type 2 nitric oxide synthase
Chronic obstructive pulmonary disease	Pro-inflammation Reduction of lung function
Asthma	Pro-inflammation
Psoriasis	Pro-inflammation
Cognitive impairment	Pro-inflammation of glia cells
Systemic lupus erythematosus	Pro-inflammation and atherosclerosis
Type 1 diabetes	Dual effect (pro-inflammation and anti-inflammation)
Pancreatitis	Protection and anti-inflammation
Rheumatoid arthritis	Pro-inflammation in animals Prevention of erosive arthritis
Acute colitis	Protection and anti-inflammation

In human studies on multiple sclerosis (MS), it has been reported that the secretion of leptin is increased in both the serum and the cerebrospinal fluid (CSF) of naive patients with MS. This positively correlates with the secretion of IFN- γ in the CSF, and inversely correlates with the percentage of circulating T_{reg} cells. Moreover, the increase of leptin in the CSF is higher than in the serum, suggesting possible secondary *in situ* synthesis of leptin in the CNS and/or an increased transport across the blood-brain barrier following enhanced systemic production.

Patients affected by Crohn's disease exhibit increased visceral adiposity with hypertrophic mesenteric fat, independent of the body mass index. In these patients, it was observed an overexpression of both leptin mRNA and leptin protein by the hypertrophic mesenteric fat, suggesting an inflammatory role of leptin.

In rheumatoid arthritis, leptin plays an important effect by increasing Th1 cytokine expression. In experimen-

tal models of arthritis, *ob/ob* and *db/db* mice exhibited less severe disease, with lower levels of IL-1 β and TNF- α in the synovial liquid, and T cells presented decreased proliferative response induced by the antigen and a shift toward a Th2 cytokine profile. On the other hand, clinical studies have reported controversial results when addressing the relationship between plasmatic and synovial leptin levels versus the activity or severity of the disease.

Leptin is involved in the pathogenesis of osteoarthritis via the production of matrix metalloproteinases and type 2 nitric oxide synthase. Moreover, the cartilage of patients with osteoarthritis present higher production of leptin. Also, high leptin levels have been reported in patients with systemic lupus erythematosus in whom the elevated leptin levels were positively correlated with atherosclerosis and inflammatory biomarkers of atherosclerosis.

Leptin levels increased just before the commencement of hyperglycemia and diabetes in spontaneous autoimmune non-obese diabetic (NOD) mice, a model of type 1 diabetes. In addition, exogenous administration of leptin to these animals enhanced interferon- γ production by T-cells, and expanded the autoimmune destruction of β -cells, suggesting a possible pro-inflammatory influence of leptin on Th1 cells in this disease. Children with greater weight gain in the first year of life were more prone to develop type 1 diabetes in concordance with the evidence that overnutrition and increased postnatal growth may be related to development of autoimmunity.

High levels of leptin were reported during acute exacerbations of chronic obstructive pulmonary disease, and were associated to serum IL-6 and TNF- α . Leptin is important to airway hyperresponsiveness associated with obesity, by the stimulation of cytokine and chemokine production in the lungs, but just in conjunction with other inflammatory agents and in supraphysiological levels.

Leptin may also be important in the cognitive impairment observed in obesity and high fat diet, by means of inflammatory and oxidative pathways in microglia cells. Finally, it been suggested that leptin may be a link between adipose tissue and psoriatic inflammation. References (11,55,56) summarize the roles of leptin on different chronic inflammatory diseases.

Leptin as an anti-inflammatory cytokine

Leptin may be protective against type 1 diabetes by reducing virus-induced insulinitis (57). Besides, exogenous leptin may exert an anti-inflammatory role in the exo-

crine pancreas, protecting rats against acute pancreatitis mediated by the nitric oxide pathway (58).

Leptin also played an anti-inflammatory effect in an experimental model of acute colitis via a neutrophil-dependent mechanism that depends on the HPA (hypothalamic-pituitary-adrenal) axis (59). Another study showed that endogenous leptin may locally protect the joints from the most severe form of erosive rheumatoid arthritis in humans (60). Table 2 summarizes the role of leptin in clinical conditions.

CONCLUSIONS

Leptin plays a major role in the regulation of the immune system. It has a positive effect on thymocytes, leading to an overall increased level of T cells, and inhibits the transformation of naive T cells into Th2 cells, which are anti-inflammatory. Additionally, leptin increases macrophage and monocyte proliferation rates, thereby increasing the levels of inflammatory cytokines (TNF- α , IL-1, IL-6) (Figure 5). The absence of leptin leads to immune defects that ultimately translate into increased mortality due to infections. It is also a major regulator of inflammatory response, with mainly pro-inflammatory actions. In excess, commonly seen in states of excess adiposity, leptin has been associated with several diseases where inflammation plays an important role in morbidity, such as cardiovascular diseases, rheumatoid arthritis, and cancer.

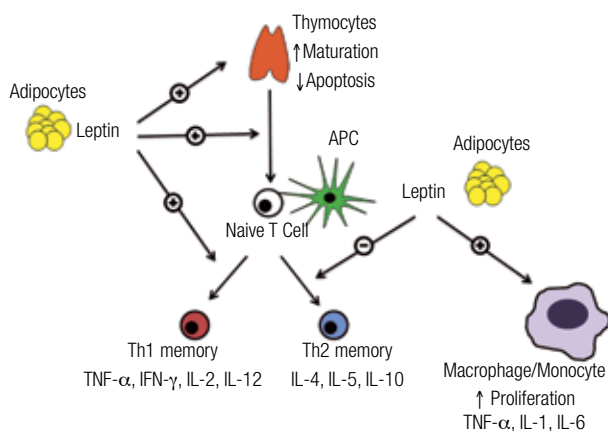


Figure 5. Leptin and the immune system.

Leptin has a positive effect on thymocytes by increasing rates of maturation and decreasing rates of apoptosis, leading to an overall increase in the level of T cells. When naive T cells interact with antigen-presenting cells (APC), they can differentiate into T-helper cell type 1 (Th1) or type 2 (Th2). Leptin inhibits the transformation of naive T cells into Th2 cells, which are anti-inflammatory. Additionally, leptin also increases macrophage and monocyte proliferation rates, thereby increasing the levels of inflammatory cytokines (TNF- α , IL-1, IL-6). Adapted from (11).

Leptin acts by activating its receptor, which in turn triggers different molecular pathways, namely the JAK-STAT, PI3K, and ERK/MAPK pathways. Within these pathways, several molecules modulate their activation, such as SOCS3. Better understanding of the molecular mechanisms by which leptin regulates immunity and inflammation might lead to the development of therapeutic targets to treat diseases associated with leptin deficiency or excess.

Disclosure: no potential conflict of interest relevant to this article was reported.

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