Leptin Action in the Thymus

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Leptin was first characterized as a hormone that plays a central role in the control of body adiposity. A number of studies later revealed several other functions for leptin, including the capacity to modulate immune system activity. Currently, leptin occupies an important position as a unifying mechanism integrating nutritional status and immune function. Here, we will review some of the actions of leptin in the immune system, with special attention to the functions it exerts in the thymus.

Key words: immunodeficiency; obesity; malnutrition

Introduction

Specificity and diversity are two of the most remarkable features of the adaptive immune system.1 Both these properties result from the adequate function of the thymus generating and selecting T lymphocytes that compose the immune repertoire.2 Thymic function depends on the complex interactions between the anatomic and histological structures of the organ and a number of immune, endocrine, neural, and nutritional factors.1,3

One of the most ancient and eventually empirical concepts in medicine is that malnutrition is strongly related to defective immune function.3,4 In fact, not only malnutrition but also another extreme nutritional dysfunction, obesity, are currently known as predisposing conditions for different forms of immune disorders ranging from severe forms of immunodeficiency to an increased risk of autoimmunity.5 Following the discovery of the primarily adipostatic hormone leptin in 1994,6 a number of additional important functions have been attributed to this peptide.7 One of the most remarkable functions is the capacity of leptin to modulate immune system activity.8 Both animal models and humans with defective leptin function present important features of immunodeficiency, which include anomalous activity of the thymus and thymic atrophy. Since leptin is produced in direct proportion to body adiposity, its concentration in the blood reflects the nutritional status at a given time.9 The hypothalamus is one of the most important sites of action for leptin. In this organ, leptin acts in concert with insulin to modulate food intake and thermogenesis and, therefore, to control the body’s stores of energy.9–11 However, under certain environmental conditions, insufficient or excessive food consumption leads to pathological adiposity and, therefore, to anomalous leptin production. In malnutrition, leptin levels are extremely low12 as are its functions. Conversely, in obesity, leptin levels are high, but at least some of its functions are impaired as a result of a phenomenon of molecular and functional resistance to its activity.13,14

As such, leptin acts simultaneously to control energy homeostasis and immune function; its activity can be impaired in malnutrition, from defective production, and in obesity, from molecular and functional resistance. Thus, a central role for leptin as a unifying mechanism to integrate the nutritional status and the
immune system has been proposed. Here, we will review some of the most important advances in this field, paying special attention to the actions of leptin in the thymus.

**Leptin and Leptin Receptor**

Leptin, the product of the *ob* gene, is a 16-kDa protein produced predominantly by the white adipose tissue in direct proportion to whole body adiposity. While initial reports regarded leptin solely as a hormone, further characterization of its structure and the identification of its receptor, the ObR, revealed that it possesses more features of a cytokine than of a hormone. In fact, the resolution of leptin’s tertiary structure showed a molecule composed of four interconnected anti-parallel α-helices, which share high similarity with the cytokines interleukin (IL)-6, IL-11, IL-12, and granulocyte colony-stimulating factor. Nevertheless, with regard to its multiple actions in distinct organs and tissues, leptin behaves as a hormone. Therefore, the creation of a new terminology, adipokine, to designate leptin and the other adipose tissue-derived substances that were identified following leptin’s first appearance proved welcome.

The *ob* gene is regulated by C/EBP-α (ccaat/enhancer binding protein-alpha), glucocorticoid responsive element, CCATT/enhancer, and specificity protein-1. Hormones, such as glucocorticoids, testosterone, and insulin, as well as nutrients, such as fatty acids, glucosamine, and malonyl-coenzyme A, which are known to modulate leptin expression, provide further support for the endocrine and metabolic roles of this peptide. In addition, leptin expression is regulated by immunological stimuli, such as lipopolysaccharide, tumor necrosis factor-α, IL-6, and IL-1β, which illustrates its integration with the immune system.

In the bloodstream, leptin can bind to macromolecules or circulate in a free form. The ratio of free/bound leptin plays a role in its biological activity. Free leptin has direct access to receptors, but, because of proteolytic cleavage, its half-life is short. Conversely, protein bound leptin has an impaired bioavailability but a longer half-life. As a peptidic signaling molecule, leptin acts through a transmembrane receptor, the ObR (Fig. 1), which belongs to the type-I cytokine receptor family because of its high similarity with the receptors for IL-2, IL-3, IL-4,
IL-6, IL-7, leukemia-inhibitory factor, granulocyte colony-stimulating factor, prolactin, growth hormone, and erythropoietin. All receptors of this class are transmembrane proteins that transduce the incoming signal through the activation of an associated tyrosine kinase. The ObR is encoded by the db gene, and as a result of alternative splicing at least six isoforms of the receptor can be generated: ObRa-ObRf. All forms of the receptor share the extracellular domain. The shortest isoform, ObRe, is a secreted form of the receptor, which is found in the bloodstream and plays a role in the control of leptin’s biological activity. The remaining forms are membrane bound and exert different roles in signal transduction. The largest isoform, ObRb, is implicated in most of leptin’s actions because its intracellular portion contains various motifs required for interaction with downstream components of the leptin signaling pathway.

**Leptin Signal Transduction**

The tyrosine kinase JAK2 (Janus kinase 2) is the most important protein involved in the transduction of the leptin signal in target cells. Upon leptin binding, ObRb undergoes dimerization, which accompanies JAK2 autophosphorylation and the tyrosine phosphorylation of two residues (Tyr985 and Tyr1138) in the receptor. These events lead to the activation of at least three distinct intracellular signaling pathways. Tyrosine phosphorylation of Tyr985 recruits the tyrosine phosphatase SHP2 (Src homology 2 domain-containing protein tyrosine phosphatase), which mediates the activation of the p21ras/extracellular signal-regulated kinase signaling pathway. Tyrosine phosphorylation of Tyr1138 recruits signal transducer and activator of transcription (STAT)3 to the ObRb, leading to STAT3 tyrosine phosphorylation and translocation to the nucleus, providing a rapid path for leptin-induced control of gene expression. Finally, the autophosphorylation of JAK2 leads to the recruitment and tyrosine phosphorylation of insulin receptor substrate (IRS)1/2 adaptor proteins, which promote the activation of phosphoinositide (PI3)-kinase and its downstream signaling. It is possible that several other signaling pathways are also activated through the ObRb. These may include other substrates for JAK2, as a large number of Tyr residues may be phosphorylated following kinase activation, and the engagement of as yet unknown tyrosine kinases because signal transduction through IRS1/PI3-kinase/Akt can occur even in the absence of JAK2 activation.

**The Thymus in Animal Models of Defective Leptin Activity**

There are three animal models frequently employed to evaluate the outcomes of defective leptin activity. The ob/ob mouse is a monogenic model with a defect in the ob gene that leads to no production of leptin. The db/db mouse and the Zucker rat have truncated and nonfunctional forms of the ObR from monogenic defects of the db gene. All three models were developed long before 1994, the year when leptin was first described, and from the very beginning of their characterization, evidence for thymic dysfunction was noticed.

Reduction of thymic size is a common feature of all three models. In the Zucker rat, the thymus is about 30% smaller than in the lean control, while in ob/ob and db/db mice the difference may reach up to 50%. As ascertained in db/db mice, this precocious atrophy of the organ is accompanied by an incipient depletion of T cells in the cortex and an increased number of Hassall’s corpuscles. The epithelial cells present an increased number of vacuoles and granules of different aspects at the ultrastructural level. Functionally, there is a precocious age-dependent decrease in the intrathymic contents and serum levels of the thymic epithelial cell-derived hormone thymulin.
Besides anatomical and structural differences, the thymi of obese mice present a considerably different distribution of thymocyte subpopulations compared to lean mice. The absolute number of thymocytes are reduced by about 10-fold, which is accompanied by a relative increase in CD4\(^-\)CD8\(^-\) and CD4\(^+\)CD8\(^-\) cells and by a relative decrease in CD4\(^+\)CD8\(^+\), resulting in a decreased ratio of CD4\(^+\)CD8\(^+\)/CD4\(^-\)CD8\(^-\). Moreover, thymocytes from obese mice proliferate significantly less than cells from lean-mice counterparts when exposed to unspecific stimulus.

### Immunological Features of Humans with Defective Leptin Function

There are only a few reported cases of defective leptin function in humans. These patients present extreme obesity from early in life and some features of immunodeficiency. Some of these individuals report frequent upper respiratory tract infections. The relative and absolute numbers of CD4\(^+\) cells in peripheral blood are reduced, while CD8\(^+\) and CD19\(^+\) are increased. The ratio CD4\(^+\)/CD8\(^+\) is about 0.5 (normal 1.0–2.6). In addition, the in vitro proliferative response of peripheral lymphocytes from leptin-deficient patients is severely compromised.

### Effects of Leptin in the Thymus

Leptin plays an important role in thymic mass maintenance. In acutely starved mice (48-h fasting) the relative mass of the thymus decreases up to 60%, an effect that is completely suppressed by leptin treatment. In addition, in ob/ob mice an increase in thymic cellularity, as high as 18-fold compared to untreated animals, is achieved with chronic leptin infusion. Apparently, both direct and indirect mechanisms take place in this regulation. The modulation of glucocorticoid levels by leptin and by changes in body adiposity seems to be one of the most important indirect mechanisms involved in this regulation. However, leptin acts directly, both acutely and chronically, to inhibit apoptosis in the thymus, and this may be one of the most remarkable features of leptin as a factor that controls immune function.

In order to exert its apoptosis-controlling effects in the thymus, leptin depends on the presence of the long form of the leptin receptor. Upon ligand binding, the ObRb undergoes tyrosine phosphorylation, which is dependent on at least one associated tyrosine kinase, JAK2. Following JAK2 activation, the leptin signal generated intracellularly is rapidly driven to the control of gene expression by the engagement and activation of STAT3. In addition, leptin signaling in the thymus leads to IRS1 phosphorylation and the activation of PI3-kinase activity, which induces the activation of the serine kinase Akt. Therefore, at least some of the canonical steps of leptin signal transduction, originally described in the hypothalamus, are present and active in the thymus. However, in contrast to its actions in the central nervous system, at least one event controlled by leptin in the thymus is not dependent on JAK2 activation. The chemical inhibition of JAK2 activity does not modify the capacity of leptin to inhibit thymic apoptosis. Interestingly, inhibiting IRS1 expression or PI3-kinase activity completely suppresses the anti-apoptotic effects of leptin in this organ. Therefore, it is expected that another, hitherto unknown, kinase links the ObRb to the IRS1/PI3-kinase/Akt signaling system and controls apoptosis through this path (Fig. 1).

Last, it remains to be defined whether leptin acts upon the thymus only through an endocrine circuit or if there is an intrathymic production of this molecule. As previously reviewed, several peptidic hormones, classically known to be produced in endocrine glands, are also produced in the thymus. Moreover, preliminary data derived from microarray analyses indicate a constitutive expression of leptin by the human thymic epithelium (L. Lacerta and W. Savino, unpublished data). Thus,
one can conceive of a paracrine effect of leptin upon the thymus, and this hypothesis represents an interesting point that deserves further investigation.

Concluding Remarks

Unraveling leptin action in the immune system is helping to progress the characterization of the mechanisms involved in the common association between nutritional dysfunctions and anomalous immune activity. Moreover, even in primary immunodeficiency or in HIV-infected hosts, which also exhibit a severe thymic atrophy, leptin serum levels are low, suggesting that not only leptin modulates immune function but also the immune system can exert some control in leptin production. Advances in this field may reveal novel targets for therapeutics of distinct forms of immune diseases.

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Conflicts of Interest

The authors declare no conflicts of interest.

References


