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Opinion

Leptin Regulation of Immune Responses

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Leptin is a regulatory hormone with multiple roles in the immune system. We favor the concept that leptin signaling 'licenses' various immune cells to engage in immune responses and/or to differentiate. Leptin is an inflammatory molecule that is capable of activating both adaptive and innate immunity. It can also 'enhance' immune functions, including inflammatory cytokine production in macrophages, granulocyte chemotaxis, and increased T_h17 proliferation. Leptin can also 'inhibit' cells; $CD4^+$ T cells are inhibited from differentiating into regulatory T cells in the presence of elevated leptin, while NK cells can exhibit impaired cytotoxicity under the same circumstances. Consequently, understanding the effect of leptin signaling is important to appreciate various aspects of immune dysregulation observed in malnutrition, obesity, and autoimmunity.

Trends

Leptin signaling can regulate innate inflammatory responses, such as cytokine production in macrophages and mast cells, as well as leptin-mediated chemotaxis in granulocytes.

Leptin signaling can regulate adaptive immunity. It is required for T_h17 differentiation through upregulation of transcription of RORγt.

Leptin signaling can suppress regulatory T cell (Treg) differentiation.

Inhibition of the leptin receptor blocks macrophage microbicidal and phagocytic functions, as well as the maturation of dendritic cells.

Leptin can inhibit natural killer (NK) cell activation under certain circumstances, a unique effect not observed in other cell types.

The Leptin Hormone: An Indicator of Energy

Leptin is an important signal of energy availability. It has a role in metabolism by signaling satiety, and is often paired with the hormone ghrelin, acting as a counter to the increased hunger effect mediated by ghrelin. However, ghrelin is dynamic, with peaks and dips reflecting an individual's hunger and satiety [1,2]. Leptin levels vary diurnally, but exist at a steadier level overall than those of ghrelin [3]. Furthermore, when exogenous leptin is administered, it does not cause satiety, but reduces eating to normal levels in obese mice [4,5]. Leptin concentrations are dramatically altered with nutritional dysfunction; indeed, high and low concentrations are seen in obesity and malnutrition, respectively [2,6]. Therefore, leptin is considered an energy indicator, signaling when sufficient energy is available for the metabolism of an organism.

Nutritional disruption of leptin signaling is common. Malnutrition results in hypoleptinemia, while obesity results in hyperleptinemia. These outcomes can have opposite effects on immune cells, which can be partially attributed to leptin function. For instance, studies of malnourished children reported qualitative differences in cytokine production, where reduced levels of certain cytokines were observed [7–10]. *In vitro* studies have also shown that human T cell activation and cytokine production can be induced or 'rescued' upon exogenous incubation with leptin following nutritional rehabilitation [7,11]. In contrast to malnutrition, upregulated inflammatory responses have been typically observed with obesity.

Recent discoveries have uncovered greater detail about how leptin regulates the immune system. Research into the effects of elevated leptin in biology has solidified the concept that leptin is a powerful proinflammatory molecule that is responsible not only for the upregulation of cellular functions, but also for the differentiation of immune cell lineages into proinflammatory subsets. Additionally, conditions of hypoleptinemia have shown that normal leptin is required for full functionality. Here, we discuss some of the latest advances in leptin regulation and immunity, at an urgent time when treatments are being sought for various conditions, including obesity and autoimmunity.

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Leptin Receptor Signaling in the Immune System

Leptin Receptor Signaling

The leptin receptor (LepR) is ubiquitously expressed on the surface of immune cells, both peripheral and bone marrow-derived cells [12,13]. There are six isoforms that arise from one coding gene, the three most common being the long, short, and soluble forms. The long and short forms of LepR are most commonly expressed on cell surfaces [14,15]. The long-form LepR contains an extracellular domain and an intracellular domain that bears a JAK2 signaling site, as well as three tyrosines (Tyr) that can be phosphorylated (see Figure I in Box 1). The short-form LepR contains only the JAK2 intracellular signaling site, which suggests that the binding of JAK2 is particularly important downstream of leptin.

The extensive signaling pathways triggered by leptin make it challenging to associate specific signaling molecules to particular leptin-mediated biologic effects. However, the JAK2–PI3K, JAK2-Tyr 985–ERK1/2, and JAK2-Tyr 1138–STAT3 pathways have recently emerged as examples of pathways by which leptin can induce immune cell activation (Box 1). Further understanding of these and other pathways may facilitate our ability to induce or prevent leptin-mediated immune activation in a context- and cell-dependent manner.

Leptin Impacts Adaptive Immunity: T Lymphocyte Function and Differentiation

Leptin Deficiency Impairs T Cell Functions

Although the mechanisms of leptin regulation of T cell function are not fully understood, leptin signaling has considerable effects on T cells, and leptin also functions as an inflammatory cytokine [12,13]. Leptin deficiency in mice and humans leads to a reduction in total $CD4^+$ T cell numbers, and a shift from Type 1 Th_1 (proinflammatory) to Type 2 helper (Th_2) T cell phenotypes [12,13]. Infection has been shown to induce increased leptin levels; the finding that acute infection coincides with increased human serum leptin has been observed particularly with bacterial infections (e.g., *Staphylococcus aureus*) and sepsis in the bloodstream [16,17]. While adipocytes are the primary leptin producers, recent evidence has revealed that leptin is also produced by other cell types (e.g., phagocytes) at sites of bacterial or parasitic infection (Box 2). As such, it is clear that leptin is implicated in the regulation of inflammatory as well as immune responses.

In T cell function, defective T cell activation and metabolism have shown in fasting mice exhibiting reduced leptin levels [18]. In one study, decreased $CD4^+$ T cell proliferation in the periphery correlated with diminished leptin concentrations [18]. Remaining T cells exhibited reduced secretion of proinflammatory cytokines IL-2 and IFN-γ following anti-CD3 and anti-CD28 stimulation *in vitro*. These effects could be rescued following the addition of exogenous leptin to cultures. Moreover, the proliferation of $CD4^+$ T cells from leptin-deficient **db/db** (or $db^{-/-}$ mice, see Glossary) was also reduced relative to wild-type cells, and the T cell defects in mice were abolished *in vivo* upon leptin injection [18]. In addition, leptin deficiency resulted in low glucose uptake in $CD4^+$ T cells, suggesting that leptin directly regulates T cell metabolism, to indicate a potential lack of available energy for activation inside a cell [18]. In murine bone marrow **adoptive transfer** experiments, effects of leptin were shown on activated T cell function and metabolism and were found to be hematopoietic cell-intrinsic. The use of T cell-specific LepR conditional knockout mice also indicated a T cell-specific leptin-signaling requirement for cytokine secretion and glucose uptake regulation [18].

In addition to its impact on T cell function, leptin also has a role in T cell differentiation; in particular, the $CD4^+$ helper T_h17 and regulatory T cell (Treg) lineages appear to be affected antagonistically by leptin signaling.

Glossary

Adoptive transfer: the transfer of a small population of immune cells from a donor into a host, most commonly via the blood stream.
Db/db mice: mice that have a genetic deficiency in the leptin receptor resulting in the absence of expression of the long-form leptin receptor on all cell types. They are obese, diabetic, and infertile.
Diet-induced obesity (DIO): most commonly used to refer to animals fed a high-fat diet to induce weight and adiposity gain.
Ob/ob mice: mice that have a genetic deficiency in leptin expression and no detectable circulating leptin. They are obese, diabetic, and infertile.
***Rag1*$^{-/-}$ mice:** mice that are immunodeficient and produce no mature B or T cells.
Q223R leptin receptor variant: a glutamine to arginine single nucleotide polymorphism in the extracellular domain of the leptin receptor, resulting in reduced leptin receptor signaling

Box 1. Leptin Receptor Signaling Pathways in Immune Cells

Leptin signaling has a range of effects on disease susceptibility through its downstream pathways. The inflammatory effect of leptin signaling within immune cells has been demonstrated; an assessment of the end-products of signaling pathways resulting from leptin binding is needed to understand leptin-mediated regulation of immune function (Figure I).

JAK2 and PI3K Pathways

JAK2 activation after leptin binding leads to activation of Akt/PI3K. PI3K then activates mTOR and p38 MAPK. This pathway has been implicated in the chemotaxis of macrophages and neutrophils towards leptin, and in lipid body formation in macrophages and epithelial cells [55–59]. mTOR signaling has also been implicated in $CD4^+$ T cell differentiation into Tregs. Leptin stimulates mTOR signaling, which, if elevated above a certain threshold (as in cases of inflammatory leptin release), results in inhibition of Treg differentiation [54]. Both mTOR and p38 MAPK signaling have been implicated in apoptotic resistance and proliferation resulting from leptin signaling in epithelial cells and neutrophils [56,59,60]. Since anti-apoptotic signaling is a common outcome of leptin signaling, this pathway likely has a ubiquitous role in many cell types.

The Tyrosine Signaling Pathways

Tyrosines (Tyr) 985 and 1138 are responsible for the remainder of known leptin signaling downstream effects, while Tyr1107 has not been implicated in any models of infection or immunosuppression, Tyr985 signals through SHP2 and ERK1/2, ultimately phosphorylating p38 MAPK. Most of the data concerning this pathway come from investigations of macrophage function. Macrophage chemotaxis has previously been shown to be equally dependent on this pathway as it is on PI3K [55]. More recently, leukotriene synthesis in macrophages was also associated with this pathway, because a Tyr985-knockout mouse model presented alveolar macrophages with reduced leukotriene synthesis [26]. It is possible that Tyr985 signaling results in the production of critical building blocks of leukotrienes.

Tyr1138 signals inhibit leptin signaling. STAT3 phosphorylation results in transcription of SOCS3, a negative feedback protein that inhibits LepR signaling by binding to Tyr985 [61]. Mice expressing a seronine instead of a tyrosine at position 1138 on the LepR signaling tail (S1138) have revealed contrasting effects of this signaling pathway. A *Staphylococcus pneumoniae* respiratory infection in S1138 mice resulted in increased leukotriene synthesis, phagocytosis, bacterial killing, and ERK1/2 activation in alveolar macrophages [62]. This is likely a result of reduced SOCS3 signaling, which in turn reduces inhibition of the Tyr985 signaling pathway. By contrast, S1138 mice infected with *Clostridium difficile* have lower levels of several proinflammatory chemokines in the intestine [36], suggesting that inflammatory signals are translated via STAT3 in addition to the inhibitory SOCS3 signal. This pathway also propagates antia-poptotic signaling. *In vitro* cell lines expressing a Tyr1138-knockout exhibit increased susceptibility to *Entamoeba histolytica* challenge along with increased cell lysis [38].

There is a large knowledge gap in what is known about the effects of leptin signaling, and the pathways that are responsible for those effects. Consequently, it is important to understand the relative contributions of signaling molecules stemming from LepR engagement, as well as the outcomes of different LepR isoform triggers, particularly under conditions of low versus high leptin concentrations.

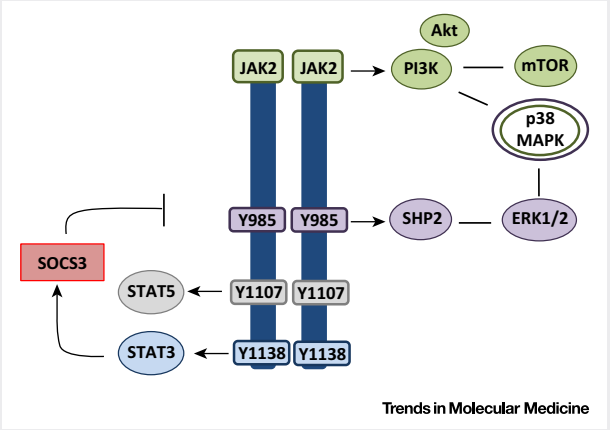

Figure I. Signaling Pathways of the Leptin Receptor. JAK2 activates PI3K, which can activate the mTOR signaling complex and p38 MAPK. Tyrosines (Tyr;Y) 985, 1107, and 1198 are phosphorylated by JAK2. Tyr985 phosphorylates SHP2, leading to phosphorylation of ERK1/2 and p38 MAPK. Tyr1107 phosphorylates STAT5. Tyr1198 phosphorylates STAT3. SOCS3 is transcribed following STAT3 phosphorylation, and results in a negative feedback loop achieved by binding to Y85 [63]. Adapted from [63].

Box 2. Leptin Is Produced as an Inflammatory Cytokine

Peripheral cells other than adipocytes can produce leptin as an inflammatory response after infection. Human and murine lung tissue taken after bacterial and viral pneumonia has shown significantly greater leptin staining in epithelial cells compared with uninjured lungs. Bronchoalveolar lavage (BAL) samples taken after endotoxin inhalation have also been shown to exhibit elevated leptin concentrations in humans [42]. Intestinal concentrations of leptin also rise in mice upon infection with *Entamoeba histolytica*, and in a cecal ligation puncture septic animal model [41,64]. Leptin production could be acting as an inflammatory enhancer, recruiting cells and boosting effector functions to combat infection.

Immune cells are also producers of leptin as an activation response. Treg and $CD4^+$ effector T cells have been observed to produce leptin after activation [20,54]. Leptin can be detected in mast cells from obese individuals and in DIO mice, as well as in human basophils from lean individuals [31,47]. Leptin production by immune cells could be acting as an autocrine and/or paracrine signal to perpetuate leptin-associated effects, because immune cells express the LepR on their surface.

T_h17 and Treg Cells: Two Poles of Leptin Signaling

Tregs are mediators of immune tolerance and suppressed inflammation, and T_h17 cells are proinflammatory cells generated to combat infections; both have been implicated in several autoimmune diseases [19].

In individuals with the autoimmune disease Hashimoto's thyroiditis (HT) who exhibit dysregulated thyroid function, increased leptin levels have been reported in plasma and within circulating $CD4^+$ T cells, relative to healthy controls [20]. In these female patients with HT, a positive association between $CD4^+$-derived leptin levels and the percentage of T_h17 cells in peripheral blood was also documented. When isolated $CD4^+$ T cells from these patients were stimulated *in vitro* with anti-CD3/anti-CD28 antibodies in the presence of a leptin-neutralizing antibody, a reduced percentage of cells were able to differentiate into T_h17 cells [20]. Furthermore, a study investigating a murine model of collagen-induced arthritis also found that leptin administration to joints resulted in earlier-onset and exacerbated arthritic symptoms, as well as an increased presence of T_h17 cells in joint tissues [21]. Thus, these and other studies have suggested an important association between leptin signaling and various forms of autoimmunity.

A recent finding reinforced the idea that LepR is necessary for $CD4^+$ T cell differentiation into T_h17 cells, demonstrating an upregulation of RORγt expression, an essential transcription factor required for T_h17 differentiation [22]. $CD4^+$ T cell specific LepR-knockout mice presented a 50% reduction in T_h17 cell numbers in the intestinal lamina propria, and were unable to clear enteropathogenic *Citrobacter rodentium* infections, which require T_h17-secreted IL-17 to promote recovery [22]. *In vitro*, LepR-knockout $CD4^+$ T cells also exhibited an 80% reduction in T_h17 differentiation. **Ob/ob** leptin-deficient mice receiving exogenous leptin exhibited a dose-dependent increase in T_h17 cell numbers, supporting the role of leptin in T_h17 differentiation [22,23]. Leptin addition to culture media also resulted in upregulation of the T_h17 transcription factor RORγt in $CD4^+$ T cells [21,23]. However, leptin did not seem to affect proliferation, because adoptive transfer of a combination of $CD4^+$ T cell specific LepR-knockout and wild-type $CD4^+$ T cells into immunodeficient ***Rag1*$^{-/-}$** mice exhibited comparable proliferation (BrdU incorporation) in the two donor populations [22].

Tregs (another $CD4^+$ T cell subset) are inhibited by leptin signaling. Comparison of obese and lean humans revealed that an obese cohort of individuals presented reduced numbers of circulating Tregs [24]. Treg numbers in the obese group were also inversely correlated with leptin and body mass index (BMI), whereas in the control healthy-weight cohort, no correlation with leptin was found [24]. This suggested that increased leptin concentrations have an inhibitory effect on Treg differentiation. Forty-eight hours of fasting in wild-type mice resulted in an increase in Treg numbers, and leptin administration in leptin-deficient ob/ob nonfasting mice led to

decreased Treg numbers. This effect appears to be a result of leptin signaling, because neither leptin administration in db/db mice nor fasting in ob/ob mice had any effect on Treg numbers [25].

Leptin Impacts Innate Immunity

The innate branch of the immune system is also affected by leptin, and recent research has uncovered important mechanisms of functional regulation. Innate cells respond to infection and also influence the adaptive response. Understanding the effect of leptin on innate immune cells should not be overlooked because it will also help advance our knowledge of leptin signaling in general, and of its role in immunity.

Leptin Promotes Macrophage and Mast Cell Inflammatory Phenotypes

In macrophages, leptin signaling impacts the phagocytic functions of the cell. Mice with a knocked-out LepR Tyr 985 exhibited no ERK1/2 signaling [26]. These mice were susceptible to *Klebsiella pneumoniae* respiratory infection, and their alveolar macrophages not only had a reduced ability to engulf and kill bacteria, but also presented diminished inflammatory cysteinyl-leukotriene levels [26]. In another report, the addition of leptin to THP-1 monocytes as well as to primary human monocytes in culture led to surface upregulation of toll-like receptor 2 (TLR2) [27]. Given that TLR2 signaling can result in lipid body formation, which is important for cell membrane formation and leukotriene production [28,29], it is possible that leptin signaling could indirectly promote phagocytosis via TLR2 induction.

Leptin signaling also promotes proinflammatory cytokine signaling. Human mononuclear cells enriched for monocytes and incubated with leptin have shown increased secretion of TNF-α, IL-6, IL-1β, and restin *in vitro* [30]. In this study, the effect of insulin and glucose on cytokine production was examined, showing that leptin, or a combination of leptin and insulin, resulted in increased monocyte secretion of IL-6 and IL-1β. Thus, leptin signaling appears to be required in the maintenance of phagocytic functions, with the capacity to promote the production of inflammatory mediators by monocytes and macrophages.

Macrophages are also indirectly regulated by leptin through mast cell (MC) signaling. A recent report demonstrated that bone marrow-derived MCs from mouse lean white adipose tissues exhibited reduced leptin expression [31]. Leptin generated an inflammatory phenotype in MCs through induction of IFN-γ and suppression of IL-4 [31]. This in turn induced polarization of macrophages into an M1 inflammatory phenotype. By contrast, in ob/ob mice, leptin-deficient MCs exhibited anti-inflammatory properties and drove macrophage polarization to an M2 phenotype; *in vitro* co-cultures of MCs with bone marrow-derived macrophages (BMDM) increased arginase-1 and IL-10 expression following IL-4 stimulation, and suppressed inducible nitric oxide synthase (iNOS) and IL-6 expression following lipopolysaccharide (LPS) stimulation. Conversely, **diet-induced obese** (DIO) mice presented adipose tissue with an abundance of M1 macrophages. Adoptive transfer of ob/ob bone marrow MCs into DIO mice resulted in a reduction of M1 macrophages and an increase in M2 macrophages in white adipose tissue. This was also accompanied by a reduction in fat deposits [31]. Leptin-deficient MCs expanded *ex vivo* could blunt the diet-induced obesity in mice. As such, MCs appear to have important roles in obesity by responding to the inflammatory milieu associated with elevated leptin levels.

Leptin Affects Dendritic Cell Maturation and Function

Dendritic cells (DC) activate T cells after sensing inflammatory stimuli. One report suggests that leptin deficiency impairs DC maturation [32]. In ob/ob mice, the expression of MHC class II and the mature DC co-stimulatory molecules CD80, CD86, and CD40 was reduced in bone marrow DCs generated after LPS stimulation in the absence of leptin [32]. IL-6, IL-12, and TNF-α levels

from DCs were also reduced, in keeping with an immature phenotype. When ob/ob and db/db mature DCs were incubated with wild-type $CD4^+$ T cells, the T cells exhibited reduced proliferation and Th_1 cytokine production, reinforcing the notion that leptin is important in DC homeostasis and inflammatory licensing [32].

While leptin could be a maturation factor, it might also act as a priming signal preparing DCs to respond more quickly to antigen activation. Patients with Crohn's disease (CD) have increased fat deposits around the intestines. DCs from CD intestinal biopsies were examined in one study, and showed that blood and colonic DCs expressed increased CCR7 chemokine levels as opposed to those from healthy individuals, which expressed little-to-none CCR7 [33]. Incubation of DCs from healthy individuals in CD biopsy supernatants resulted in an increase in CCR7, which could be blocked with an anti-leptin antibody [33]. However, CD patients did not present more mature DCs than healthy individuals [33]. The study concluded that leptin might also have an important role in DC migration.

Neutrophil Infiltration in Response to Leptin Production

The role of leptin signaling in neutrophils has been debatable because only the short-form LepR is expressed on the cell surface of neutrophils [34]. However, the extensive signaling pathways emanating from JAK2 activation suggests that the short form should not be assumed to be 'silent'.

In db/db mice, acute lung injury by LPS inhalation resulted in reduced neutrophilia in the bronchoalveolar lavage (BAL), fluid compared with controls [35]. Moreover, in a *Clostridium difficile* infection model in db/db mice, flow cytometric analysis of large intestine immune populations revealed a reduction in neutrophils relative to control mice [36]. In the acute lung injury model, reduced neutrophilia was associated with attenuated respiratory damage in db/db mice, while in the db/db *C. difficile* murine model, a higher burden of infection was described, albeit with mitigated weight loss and a slightly improved clinical score [35,36].

The LepR variant **Q223R** results in an increase in susceptibility to enteric pathogens in humans [36–39]. A murine equivalent of this mouse exhibits increased susceptibility to the enteric pathogen *Entamoeba histolytica*, similarly to humans [40]. Flow cytometric analysis of the immune populations at the cecal *E. histolytica* infection site after a short duration indicated that infiltrating neutrophil populations were reduced in mice expressing the Q223R polymorphism [41]. Leptin regulation of neutrophil infiltration appears to be a chemotactic effect because neutrophils can migrate towards leptin. Murine neutrophils expressing the Q223R LepR variant have been shown to exhibit reduced chemotaxis towards leptin, while neutrophils from human volunteers and wild-type C57BL/6 mice have shown dose-dependent chemotaxis towards leptin [41,42]. However, one study did not find any neutrophil responses to leptin, including chemotaxis [43]. This discrepancy was possibly due to differences in methodology, because different concentrations of leptin were used (e.g. physiological vs other). *In vivo* examples of leptin elevation indicate that neutrophils do respond to leptin: diet-induced obesity in rats has been associated with increased production of peripheral neutrophils [44]. In addition, neutrophils from obese humans display increased random migration and chemotaxis towards fMLP before and after stimulation with N-Formylmethionyl-leucyl-phenylalanine (fMLP) [45]. Additionally, in an *Escherichia coli* pneumonia murine model, addition of exogenous leptin led to increased neutrophil numbers in the BAL fluid, an observation replicated in healthy mice [42].

Overall, the evidence for an effect of leptin on neutrophil chemotaxis and infiltration is strong. However, the effect of leptin signaling on other neutrophil functions remains unresolved. Reports disagree as to the effect of LepR signaling on the chemotactic response to other

chemoattractants with some studies reporting reduced chemotaxis towards chemoattractants, such as CXCL1 in neutrophils with impaired leptin signaling, while others report no effect [35,41,42].

Eosinophils and Basophil Responses to Leptin

As with neutrophils, leptin is also a chemoattractant for eosinophils and basophils. Human eosinophils and basophils isolated from human peripheral blood have been reported to migrate towards leptin in a dose-dependent manner [46,47]. *In vitro* incubation of eosinophils with leptin has also been shown to enhance chemotaxis towards eotaxin [48]. Eosinophils isolated from obese individuals have demonstrated higher chemotaxis towards eotaxin and RANTES (CCL5) when compared to lean subjects [48]. Basophils have also shown to exhibit similar increase in chemotaxis towards eotaxin after incubation with leptin [47]. This study reported that leptin was able to induce a small amount of degranulation from human basophils, but when leptin was administered in combination with a CRA-1 antibody to engage FcεRI, the basophils exhibited enhanced degranulation [47]. Thus, leptin appears to be a potent stimulus for eosinophils and basophils.

The signaling mechanism leading to enhancement of chemotaxis is unclear. In eosinophils, expression and downstream signaling of CCR3 (the receptor for eotaxin) were not increased after incubation with leptin [46]. However, actin polymerization and calcium flux were increased, suggesting that leptin promoted cellular changes independently from CCR3 signaling. Further investigations may shed some light on this point.

Leptin stimulation of human basophils has also resulted in increased secretion of type 2 cytokines IL-4 and IL-13 in these cells [47], although leptin has been traditionally associated with promoting inflammatory Th_1 cytokines [4]. However, increased type 2 cytokine production may not be surprising under certain conditions, if leptin does act as a general inflammatory mediator. This is an area that could be actively pursued.

Leptin Signaling and NK Cell Inhibition

The inflammatory nature of leptin has been demonstrated in the almost universal upregulation of inflammatory functions in immune cells. However, natural killer (NK) cells might be inhibited in their inflammatory properties as a result of prolonged increased leptin signaling [49]. In obese humans, NK cells express reduced surface TRAIL, a cytolysis ligand, as well as CD107a, a marker of degranulation and NK activity [50]. A comparison of NK cells from adult males before and after weight loss indicated that, following weight loss, NK cells produced more IFN-γ, a critical effector cytokine [51].

In vitro research into the effect of leptin on NK cells remains inconclusive because the concentration of leptin used and the time of incubation can affect the outcome. For instance, brief addition of leptin to primary human NK cells or to the cell line NK-92 was reported to result in increased perforin, TRAIL, and IFN-γ expression, with increased cytotoxicity mediated through cellular conjugation [49,52]. However, extended incubation of human NK cells with leptin for 3–4 days has been was reported to result in reduced cytotoxicity against tumor cell lines and reduced IFN-γ production [49]. Additionally, incubation with 100 ng/ml of leptin for 1–2 days, a concentration observed in obese individuals, was also reported to lead to a decrease in cytotoxicity and perforin production from NK cells [51,52].

Leptin signaling in NK cells could be operating on a threshold system. Physiologically normal concentrations could maintain normal NK effector mechanisms, with brief increases in leptin boosting effector function, but long term, suppressing NK cell functions. *In vivo* data may help resolve the discrepancies in leptin-mediated mechanisms that lead to NK cell effector suppression.

A Proposed Model of Leptin Regulation of Immune Cells: Licensing, Enhancement, and Inhibition

We propose that leptin could be regarded as: (i) a 'licenser' of immune function; (ii) an 'enhancer' of immune response mechanisms; and (iii) an inhibitor of immune function (Figure 1). A 'license'

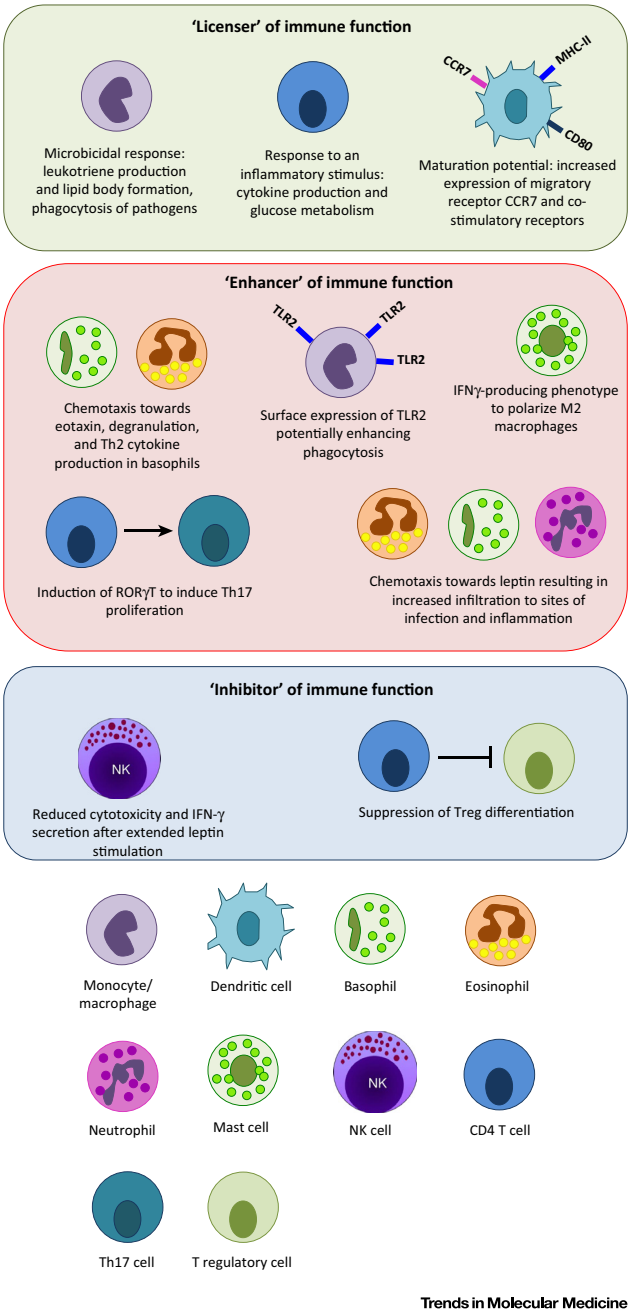

Figure 1. Proposed Model of the Effects of Leptin on Immune Cells. Current research has suggested three outcomes of leptin signaling: licensing of basic function, enhancement of effector functions, and inhibition of cell functions. Models of hypoleptinemia or reduced receptor signaling have revealed functions that require leptin to operate at adequate and 'normal' levels, suggesting a licensing effect of leptin. In instances of hyperleptinemia, such as diet-induced obesity and inflammation, leptin appears to promote or enhance the inflammatory response mechanisms of immune cells. Finally, leptin has some inhibitory effects on cellular function and differentiation, including the cytolytic potential of natural killer (NK) cells and the paths of regulatory T cell (Treg) differentiation, respectively.

to react to inflammatory signals could occur in response to a leptin signal of 'normal available energetic capacity'. 'Licensing' as such, has been reported for T cell activation and cytokine production, macrophage lipid body and leukotriene production, and DC maturation [18,26,32]. These cell types have exhibited reduced responses to infection or stimulation in models of reduced leptin signaling, suggesting that leptin is required for normal cellular function.

The 'enhancement' effects of leptin occur as part of the inflammatory role that leptin signaling has in response to infection or damage. In this context, elevated leptin has the effect of boosting certain functions, such as chemotaxis towards eotaxin for eosinophils and basophils, and basophil degranulation [46–48]. Leptin also directly induces inflammatory responses in the absence of other stimuli, because it acts as a chemoattractant for granulocytes [41,42]. $CD4^+$ T cell differentiation in T_h17 cells is a striking example of leptin signaling promoting an inflammatory effect, as is the inflammatory mast cell phenotype that polarizes macrophages to the M1 lineage [53].

The 'inhibitory' effects of leptin are an intriguing development. Inhibition of Treg differentiation is in line with the proinflammatory effects of elevated leptin signaling. By contrast, NK cell inhibition is puzzling. NK cells appear to depend on either very high concentrations of leptin, as seen with Treg suppression, or on prolonged stimulation. The mechanisms of suppression in both cell types are unclear, although the mTOR pathway may have an important role in Tregs [54].

Leptin Research: Where Are We Now?

Leptin is no longer confined to the realm of metabolism; the many pleotropic effects of the leptin-signaling pathway and the ubiquitous expression of LepR, underlie the broad impact of leptin on cellular function. Leptin could be revealed as a powerful licenser and enhancer of immune functions. Recent research with DIO murine models and with primary cells from obese humans has elevated our understanding of the role of leptin as an inflammatory cytokine. Combining these findings with the immune phenotypes of hypoleptinemic animals and cell culture models has suggested the possibility of a gradient model for leptin effects (Figure 2), where leptin might progress from a licensing to an enhancer molecule based on its concentration.

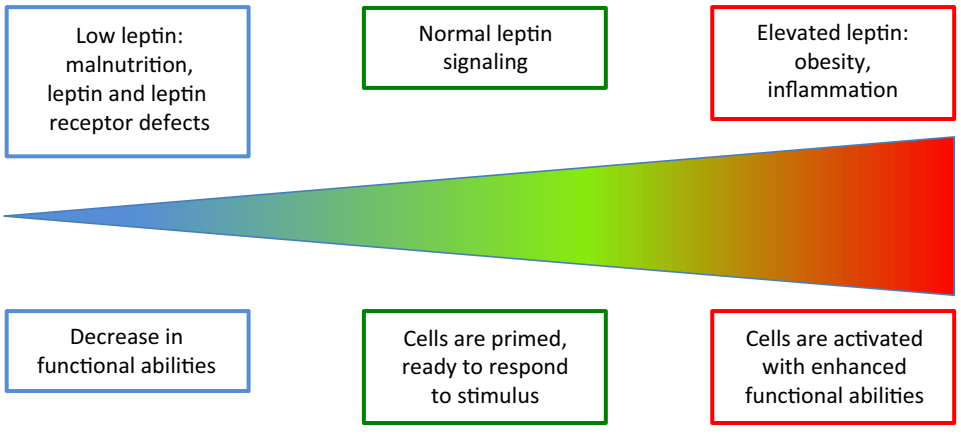

Figure 2. The Regulation Gradient of Leptin. Hypothetical model of how a leptin gradient would achieve differential regulation. Regulation might begin with the ability of cells to respond (licensing to indicate energy availability for normal inflammatory processes) and increase to determine the magnitude of a response (most often in the form of enhancement).

Concluding Remarks

We propose that leptin has effects on licensing, enhancement, and inhibition of immune cells in response to the surrounding leptin environment. Advancing our understanding of leptin signaling has clear clinical relevance. Malnutrition and obesity are nutritional disorders with different immune phenotypes and different morbidities. Understanding immunometabolic processes in obesity are is crucial, as these they likely include various mechanisms that depend on the enhancement and inhibition effects of leptin signaling. Elevated leptin can also be indicative of an infectious challenge. Future work will need to determine whether leptin has a critical role in the described processes in humans (e.g., with leptin deficiency).

The role of leptin signaling in specific cell types, such as neutrophils and NK cells, also calls for further investigation (see Outstanding Questions). Neutrophils are a first-line defense mechanism, and impairment in a hypoleptinemic environment could have ramifications for the management of diseases associated with malnutrition. The inhibition observed for NK cells and Tregs is also a fascinating avenue of research, and could represent opportunities for cancer and autoimmune treatments. Recent research has also been predominantly focused on single cell types. While this has been critical in understanding certain specific effects of leptin, the effect of cellular crosstalk in the context of high or low leptin could provide new insights. Research into the effects on leptin on immune function will bring forth a clearer picture on the mechanisms that regulate its involvement in immunometabolism, obesity, malnutrition, autoimmunity, infection, cancer, and other yet to be identified conditions.

Outstanding Questions

Does the short-form LepR on neutrophils have a function other than that of a chemotactic receptor?

Is T cell differentiation into the Th_1 lineage dependent on DC maturation status as a result of leptin signaling, or on direct T cell leptin signaling, as seen for T_h17 differentiation?

Can human malnutrition and immunity studies replicate the ob/ob and db/db murine results, as obesity studies do for DIO models?

Are the enhanced granulocyte and monocyte and/or macrophage functions in models of elevated leptin signaling subject to leptin thresholds?

What is the mechanism of the inhibitory effect of leptin signaling observed in NK cells and Tregs, and is it relevant for other immune cell populations?

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