Review

Role of Leptin in Immunity

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Leptin, a protein hormone produced by the adipocytes, has long been recognized to regulate metabolism, neuroendorine and other physiological functions. Early findings of increased leptin production during infection and inflammation and dysregulated immune response in leptin signaling-deficient mice provide strong evidence for the involvement of leptin in the immune responses. Recent data have established the regulatory function for leptin in immunity similar to the function of a pro-inflammatory cytokine, while gene-targeting studies also demonstrated an essential role of leptin in regulating hematopoiesis and lymphopoiesis. Moreover, there has been increasing evidence that leptin is involved in the pathogenesis of various autoimmune diseases. This review discusses recent advances in understanding the role of leptin in immunity and leptin-signaling pathways involved in modulating immune homeostasis and autoimmune pathogenesis. *Cellular & Molecular Immunology*. 2007;4(1):1-13.

Key Words: leptin, leptin signaling, pro-inflammatory cytokine, immune response, autoimmune disease

Introduction

Leptin, a 16 kDa non-glycosylated polypeptide product of the *obese* (*ob*) gene, is an adipocyte-derived hormone which has long been recognized as a key factor in regulating a wide range of biological responses including energy homeostasis (1), neuroendocrine function (2), angiogenesis (3, 4), bone formation (5) and reproduction (6). In addition to its critical involvement in these physiological functions, leptin has been increasingly recognized as a cytokine-like hormone with pleiotropic actions in modulating immune responses (7, 8). Leptin has been shown to provide a proliferative signal in hematopoiesis and lymphopoiesis. Moreover, leptin can activate monocytes, dendritic cells (DC) and macrophages and stimulate them to produce Th1 type cytokines (9). We have recently demonstrated that leptin is critically involved in the maturation and survival of DC (10). Leptin also exerts activating effects on neutrophils and natural killer (NK) cells and stimulate their gene expressions (11-13). Importantly, leptin has been shown to modulate the adaptive immunity *via* enhancing T cell survival and stimulating their production of pro-inflammatory cytokines such as IFN-γ and IL-2 (14). Consistent with these findings, leptin has been implicated in obesity and type I diabetes (T1D) which is commonly associated with inflammatory and T cell-mediated responses (15). While leptin is recognized as a key factor in stimulating inflammatory response, recent evidence demonstrates a detrimental involvement of leptin in promoting the pathogenesis of various autoimmune diseases such as rheumatoid arthritis (RA), colitis and multiple sclerosis. In respect of its diverse functions in immunity, leptin has been explored as a potential target for therapeutic development in treating autoimmune diseases.

Leptin expression and its regulation

Leptin, sharing a high degree of homology among species, has been increasingly recognized for its hormonal and immune actions that link nutritional status and a variety of physiological actions with immune regulation (16). Leptin circulates both as a biologically active free form and a presumably inactive bound form by association with plasma proteins and the soluble leptin receptor isoform OB-Re (17). Leptin levels correlate closely with body-mass index (BMI) and obese individuals have high serum levels of free active leptin with reduced OB-Re levels (18). The expression of leptin is regulated by hormones and inflammatory mediators. Studies on both leptin-deficient (*ob/ob*) mice and leptin receptor-deficient (*db/db*) mice have illustrated the diverse functions of leptin, as revealed by the findings of marked abnormalities in neuroendocrine function (2, 19-21), angiogenesis (3, 4), reproduction (6, 22, 23), insulin secretion (24), wound healing (25), hematopoiesis and lymphopoiesis in

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Abbreviations: BM, bone marrow; OB-R, leptin receptor.

these mutant mice (102). These data have demonstrated a direct involvement of leptin as a potent immunomodulator in regulating the various immune responses.

Leptin, a 16 kDa protein encoded by the o*b* gene, consists of 167 amino acids (16). The structure of leptin contains four interconnected anti-parallel α-helices, which is in high similarity to members of the long-chain helical cytokines such as interleukin-6 (IL-6), IL-11, IL-12, granulocyte-colony stimulating factor (G-CSF), leukemia inhibitory factor (LIF), ciliary neutrophic factor (CNTF) and oncostatin M (OSM) (26, 27). The cytokine-like structural characteristic of leptin is implicative of its function in regulating immune response. As an endocrine hormone, leptin is synthesized mainly by adipose tissue in proportion to the BMI and the body fat mass. Under certain circumstances, leptin is produced at low levels by tissues such as intestine, placenta, mammary and gastric fundic epithelium, skeletal muscle and brain (28, 29). At normal conditions the levels of circulating leptin correlate positively with leptin mRNA and protein levels in adipose tissue (30). The regulatory elements for the *ob* gene promoter include C/EBP-α, CCATT/enhancer, glucocorticoid response elements and SP-1 binding sites (31). Leptin expression in adipocytes is induced by insulin, melanyl-CoA, ATP, glucosamine and short-chain fatty acids but is inhibited by cyclic AMP, testosterone and long-chain fatty acid (32, 33). Leptin expression is also regulated by different inflammatory immune mediators (34). Levels of leptin rapidly increase during acute infection and sepsis, consistent with the findings that leptin mRNA expression is stimulated by LPS and cytokines such as TNF-α, IL-6 and IL-1β during acute inflammatory reactions (35). These cytokines stimulate short-term release of leptin to the circulation while long-term exposure to pro-inflammatory cytokines during chronic inflammation leads to reduced leptin concentrations (36). Although the kinetics of leptin induction usually resembles that of cytokine production, some studies have not found increased levels of leptin in acute inflammatory conditions such as acute experimental endotoxaemia, newborn sepsis and HIV infection in human (37, 38). Therefore, it appears that increased leptin levels exist in some acute conditions but not in others. Moreover, leptin levels are reduced during starvation and malnutrition which are associated with impaired immune response and thymic atrophy (8, 39).

Leptin signaling transduction pathways

Encoded by the diabetes (*db*) gene, the leptin receptor (OB-R) belongs to the class I cytokine receptor superfamily including receptors for IL-2, IL-6, G-CSF, LIF, CNTF, OSM and gp130 (26, 40-42). Like other cytokine receptor family members, OB-R is internalized upon ligand binding *via* clathrin-coated vesicles into endosomes (43, 44). OB-R shares highest structural similarity and signaling capability with those of the IL-6 type cytokine receptors (26). It contains four fibronectin type III domains, four conserved cysteine residues and two cytokine-like binding motifs, Trp-Ser-Xaa-Trp-Ser, in the extracelullar region (27, 40, 45). OB-R exists in a dimeric form even in the absence of leptin and is activated upon ligand binding (46). By alternative splicing, OB-R mRNA gives rise to six different isoforms that share identical extracellular binding domain but with cytoplasmic domains of different length, which include one soluble form (OB-Re), four short forms (OB-Ra, OB-Rc, OB-Rd and OB-Rf) and one long form (OB-Rb) (42). The different isoforms have distinct biological activities (47). For example, OB-Ra is responsible for the transport of leptin across the blood-brain barrier (48) whereas the soluble OB-Re serves as a regulator of circulating leptin levels (49). Among the six isoforms, only OB-Rb is most capable of transducing its signaling function and has been shown to be of prime importance in leptin-mediated signaling (47, 50, 51). Although it is highly expressed in the hypothalamus, OB-Rb can be also found in different tissues and immune cell types including various subpopulations of T cells, B cells, DC, monocytes, neutrophils, macrophages and NK cells (9, 10, 12, 13, 51-54). The ubiquitous distribution of OB-Rb in almost all tissues is in line with the pleiotrophic function of leptin.

Intracellularly, all six OB-R isoforms contain a highly conserved proline-rich box 1 (intracellular amino acid 6-17) (55, 56) but only OB-Rb has an extended intracellular domain of approximately 300 residues (57-59) (Figure 1). The major functions of all short isoforms except OB-Re are limited to leptin transport, internalization and degradation (60), although some evidence suggests that they are capable of triggering certain signaling events (61). The long receptor OB-Rb, a fully functional receptor, does not have an intrinsic tyrosine kinase domain but its box 1 motif recruits and binds janus kinases (JAKs). The box 1 motif together with the immediate surrounding amino acids is essential for JAK activity (57, 58, 62). The extended intracellular domain in the distal part of OB-Rb is required for the induction of signal transducers and activators of transcription (STAT) signaling (61). A mutation with an insertion of a stop codon leads to the truncated short OB-R in the *db/db* mouse while a point mutation of Gln for Pro at amino acid 269 in the extracellular domain is present in the Zucker fatty (*fa/fa*) rats (63, 64). Both mutations lead to severely impaired leptin-signaling activity that is responsible for the metabolic and immune abnormalities in these animals.

Upon leptin binding, OB-Rb-associated JAK2 at the box 1 motif is activated, which then auto-phosphorylates its own tyrosine residues and phosphorylates tyrosine residues on the intracellular domain of the receptor (Tyr 974, Tyr 985, Tyr 1077 and Tyr 1138) to provide docking sites for signaling proteins containing src homology 2 (SH2) domains (Figure 1). The phosphorylated tyrosine residues Tyr 1077 and Tyr 1138 bind to the STAT proteins which are then activated and translocated to the nucleus to stimulate gene transcription. Both Tyr 1077 and Tyr 1138 bind to STAT5 while only Tyr 1138 recruits STAT1 and STAT3 (65, 66). The other two phosphorylated residues Tyr 974 and Tyr 985 recruit SH2 domain-containing phosphatase 2 (SHP2) which activates the mitogen-activated protein kinase (MAPK) pathways including extracellular signal-regulated kinase (ERK1/2), p38

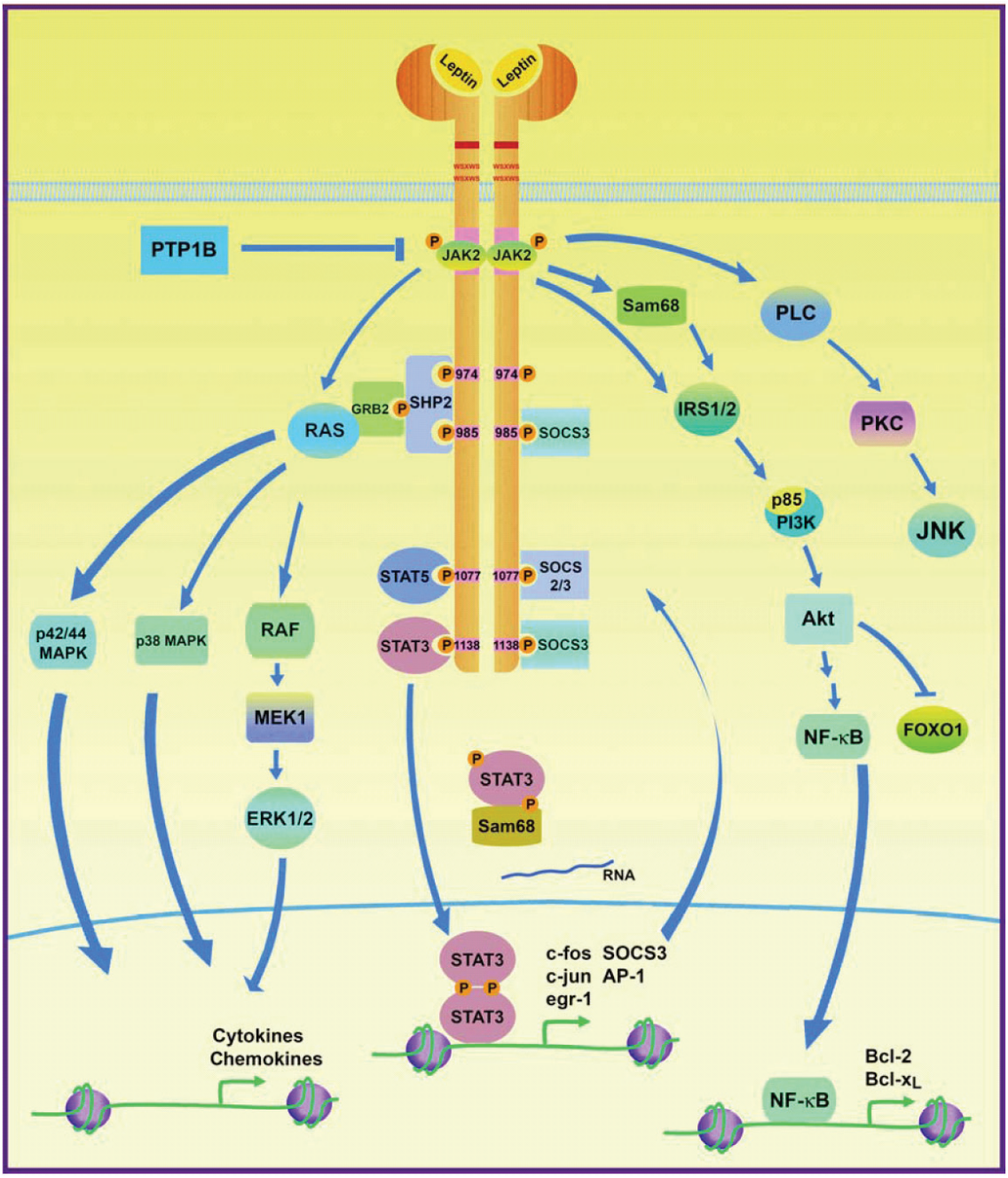

Figure 1. Signaling pathways activated by leptin. Upon leptin binding to the long isoform of the leptin receptor (OB-Rb), JAK2 at the box 1 motif is activated, which then auto-phosphorylates its own tyrosine residues and phosphorylates the tyrosine residues (Tyr 974, Tyr 985, Tyr 1077 and Tyr 1138) to provide docking sites for signaling proteins containing src homology 2 (SH2) domains. Both Tyr 1077 and Tyr 1138 bind to STAT5 while only Tyr 1138 recruits STAT1 and STAT3. STAT3 proteins form dimers and translocate to the nucleus to induce various gene expressions such as c-fos, c-jun, egr-1, activator protein-1 (AP-1) and suppressors of cytokine signaling 3 (SOCS3). SOCS3 negatively regulates signal transduction by leptin by binding to the phosphorylated tyrosines (Tyr 985, Tyr 1077 and Tyr 1138) on the receptor to inhibit binding of STAT proteins and SH2 domain-containing phosphatase 2 (SHP2). SHP2 activates the mitogen-activated protein kinase (MAPK) pathways including extracellular signal-regulated kinase (ERK1/2), p38 MAPK and p42/44 MAPK pathways through interaction with the adaptor protein growth factor receptor-bound protein 2 (GRB2) to induce cytokine and chemokine expression in immune cells. SOCS2 binds to Tyr 1077 and might potentially interfere with STAT5 binding. Src associated in mitosis protein 68 (Sam68) can form a complex with activated STAT3 upon leptin stimulation leading to its dissociation from RNA. The auto-phosphorylated JAK2 at the box 1 motif can phosphorylate insulin receptor substrate1/2 (IRS1/2) that leads to activation of phosphatidylinositol 3-kinase (PI3K)/Akt pathway. Sam68 can also be activated by JAK2 directly to phosphorylate IRS1/2 for Akt activation. Akt can regulate a wide range of targets including FOXO1 and NF-κB. Activation of NF-κB by leptin binding has been shown to induce Bcl-2 and Bcl-X_L expressions. Leptin binding to OB-Rb can also activate phospholipase C (PLC) for stimulation of c-jun N-terminal protein kinase (JNK) *via* protein kinase C (PKC).

MAPK and p42/44 MAPK pathways through interaction with the adaptor protein growth factor receptor-bound protein 2 (GRB2) (67-70). The auto-phosphorylated JAK2 at the box 1 motif can phosphorylate insulin receptor substrate1/2 (IRS1/2) that leads to activation of phosphatidylinositol 3-kinase (PI3K)/Akt and the MAPK pathways (27, 43, 66, 68, 70-72).

Leptin has also been shown to activate various isoforms of STATs including STAT1, STAT3, STAT5 and STAT6 in a variety of cell types (65, 73, 74) (Figure 1). Among various STAT proteins activated by OB-Rb, STAT3 has been most extensively reported to mediate effects of leptin on growth and function of normal and cancer cells as well as immune cells, primarily by inducing various gene expressions such as c-fos, c-jun, egr-1 and activator protein-1 (AP-1) (75-78). STAT3 has been shown to mediate the leptin signal in activating macrophages and in promoting the survival and activation of lymphocytes and peripheral mononuclear cells (53, 78-80). We have shown that STAT3 activity is impaired in the *db/db* mouse BM-derived DC. The *db/db* DC display reduced survival and maturation capacity, which is in line with previous findings that STAT3 is activated in leptin-treated human monocyte-derived DC (9, 10). Src associated in mitosis protein 68 (Sam68), an RNA binding protein involved in inhibiting cell proliferation, is tyrosine-phosphorylated and forms a complex with activated STAT3 upon leptin stimulation in human peripheral blood mononuclear cells (81, 82). The phosphorylation on Sam68 leads to its dissociation from RNA and allows it to bind to proteins containing SH2 and SH3 domains (81). On the other hand, STAT3 can induce activation of the negative-feedback regulator, suppressors of cytokine signaling 3 (SOCS3), which binds to the phosphorylated tyrosines (Tyr 985, Tyr 1077 and Tyr 1138) on the receptor to inhibit leptin signaling (83-85). The involvement of SOCS3 in the negative-feedback control of leptin signaling is suggested to underlie the development of leptin resistance commonly found in obesity (68). SOCS3 plays an important role in the negative regulation of LPS-induced CD40 gene expression by IL-10 in macrophages, which appears to mediate leptin resistance in type 2 diabetes when leptin concentrations are markedly increased in obese and diabetic individuals. Since diabetes patients are susceptible to infection, it is plausible that leptin resistance serves as one of the contributing factors for the high incidence of infection. Further studies on the role of SOCS3 in mediating the leptin effects in immune cells will provide understanding on the negative feedback regulation of leptin signaling in immunity. Other proteins of the SOCS family that are induced by leptin include SOCS1 and SOCS2. Using mammalian protein-protein interaction trap (MAPPIT), SOCS2 has been shown to interfere with the binding of cytokine-inducible SH2 protein (CIS) to Tyr 1077 of OB-Rb, which may potentially inhibit STAT5 activation (86, 87). Intriguingly, CIS transgenic mice displayed altered T cell development with a bias toward Th2 response, suggesting a potential involvement of CIS in the leptin-dependent modulation of the immune response (88). Based on the complex activation network of the STAT proteins, further analyses on their regulation by leptin signaling in immune cells will merit a fuller understanding of leptin-mediated immune modulation. Apart from the SOCS proteins induced by STATs to inhibit leptin signaling, protein tyrosine phosphatase 1B (PTP1B) is another negative regulator localized on the surface of endoplasmic reticulum and acts by dephosphorylation of JAK2 on OB-R (72, 89-91). Moreover, overexpression of PTP1B has been shown to inhibit leptin-induced SOCS3 and c-fos expression.

Leptin activates the MAPK cascade *via* the recruitment of SHP2 to OB-Rb, which then binds to GRB2 to activate further signaling steps including RAS, RAF and MEK1, leading to the activation of ERK1/2, p38 MAPK and p42/44 pathways (67-70). The primary docking site for SHP2 is phosphorylated Tyr 974 and Tyr 985 but studies have shown that OB-Rb lacking Tyr 985 is able to induce ERK signaling, although only up to a less extent (68). An alternative pathway that is independent of receptor phosphorylation for ERK1/2 activation is *via* the interaction of SHP2 and GRB2 on JAK2 (55, 92). It has been reported that leptin can induce c-jun N-terminal protein kinase (JNK) *via* phospholipase C (PLC) and subsequently protein kinase C (PKC) activation (93). Leptin stimulates the release of pro-inflammatory cytokines from human placenta and maternal adipose tissue *via* ERK1/2 and involving p42/44 MAPK and nuclear factor-κB (NF-κB) (94). In neutrophils, leptin activates chemotaxis *via* p38 MAPK pathway (95). Leptin also stimulates TNF-α production *via* p38 and JNK MAPK pathways in LPS-stimulated kupffer cells (96). Furthermore, leptin has been shown to activate the MAPK pathways to mediate anti-apoptotic effects in mononuclear cells (69, 81, 82). We previously reported downregulated p38 MAPK activity in BM-derived DC from *db/db* mice (10). The MAPK pathways are essential in regulating a wide range of immune functions. ERK1/2 and p38 MAPK pathways synergistically mediate cytokine production in DC (97) and participate in the chemokine production in CD40L-stimulated macrophages (98). With respect to the critical involvement of the MAPK pathways in immunity, further studies are required to elucidate how leptin regulates immune functions *via* these pathways.

The PI3K/Akt pathway represents a key signaling cascade which mediates effects of a wide range of ligands in a variety of different cell types. The typical target of PI3K, Akt, is an integral part of a key signaling pathway that is necessary for inducing immune and inflammatory responses (78). Akt is also the mediator of signals from pro-inflammatory cytokines and toll-like receptor ligands in a variety of immune cells. The PI3K/Akt pathway is the upstream regulator for a number of effectors including the anti-apoptotic transcription factor NF-κB. Akt inhibits its downstream target pro-apoptotic forkhead transcription factor FOXO1 or FKHR-L1, which functions to induce the expression of Bim, a pro-apoptotic member of the Bcl-2 family proteins. We have demonstrated that defective activation of various targets in the Akt signaling pathway is associated with a markedly enhanced Bim expression in BM-derived DC from *db/db* mice (10). Recently, FOXO1 is shown to directly mediate the effects of leptin in the

hypothalamus (99). Leptin binding to OB-Rb also activates some components of the insulin signaling cascade including IRS1/2 through phosphorylated JAK2 (100). The IRS proteins bind to the regulatory unit p85 of PI3K to activate the catalytic domain. The activated PI3K increases the levels of PI (3, 4, 5)P3, which stimulates phosphoinoside-dependent kinase 1 (PDK1) for the phosphorylation and activation of Akt. In peripheral blood mononuclear cell leptin can activate the p85 subunit of PI3K *via* phosphorylation of Sam68 (82). Consistent with the activating effects of leptin on Akt, an enhanced activation of PTEN, a negative regulator of Akt activity, has been detected in the *db/db* mouse BM-derived DC (10). Leptin can also stimulate human peripheral blood mononuclear cells and induce lipase activity in macrophages in response to hormone treatment *via* the PI3K/Akt pathway (69, 80). In thymic cells, leptin induces anti-apototic activity through a JAK2-independent mechanism but depends on the engagement of the IRS1/PI3K pathway (78). Together, these findings support the notion that Akt is an important mediator of leptin signaling in the immune activity.

NF-κB plays a key role in mediating different signaling systems such as JAK-STAT, PI3K/Akt and MAPK pathways to regulate the immune response. Leptin activates NF-κB in DC whereas leptin signaling deficiency leads to enhanced levels of IκB-α, the inhibitor protein of NF-κB (9, 10). Leptin stimulates the release of pro-inflammatory cytokines and prostaglandins from human placenta and maternal adipose tissue *via* NF-κB (94) and upregulates proinflammatory chemokine monocyte chemoattractant protein 1 (MCP1) in hepatic stellate cells, which is associated with enhanced severity of inflammation in mice after acute liver injury (101). NF-κB is known to regulate the expression of a variety of genes including the pro-survival factors such as Bcl-2 and Bcl-X_L, which underlies leptin signaling-mediated survival of immune cells.

Role of leptin in hematopoiesis and lymphopoiesis

The functional long isoform of the leptin receptor OB-Rb is expressed in both human and murine hematopoietic stem cells and in human B cell progenitors (102). The capacity of the BM stromal cells to express leptin provides a strong evidence for leptin in nurturing the hematopoietic stem cells. The role of leptin in regulating hematopoiesis was demonstrated by the colony formation studies where leptin addition stimulated the proliferation of stem cells and increased the numbers of lymphoid, erthyroid and myeloid colonies (102) (Figure 2). BM cells isolated from *db/db* mice showed defective colony-forming potentials in the lymphoid and myeloid lineage. Consistently, *db/db* mice showed impaired lymphopoiesis with reduced levels of $B220^+CD43^+$ pro-B cells in the BM and dramatically reduced steady-state levels of peripheral B cells and $CD4^+$ T cells, suggesting a direct role of leptin in enhancing the proliferation and expansion of hematopoietic stem cells and lymphoid progenitors. However, a recent report indicated an indirect effect of leptin on lymphopoiesis *via* the microenvironment since *db/db* BM cells transferred into wild-type recipients showed normal lymphocyte populations with functional cellular and humoral immune responses (103), while both wild-type thymus weight and cellularity were decreased when grafted into *db/db* recipients. These observations indicate that environmental factors normalized immune defects of *db/db* lymphocytes, and leptin signaling in the environment is required for normal T cell development (103). Thus, the expression of the leptin receptor on BM stromal cells as well as hematopoietic cells suggests that leptin can regulate the immune cells directly and indirectly *via* the BM microenvironment. The development and survival of B lymphocytes and other lineages in the BM is controlled by both cell-autonomous mechanisms and signals from the microenvironment (104-108). BM stroma produces a variety of cytokines that are critical to the maintenance and differentiation of the hematopoietic progenitors such as IL-7, IL-6, IL-15, Flt3L, GM-CSF and SCF, etc (108). Apart from the necessary cytokines that are produced by the BM stroma, the adhesive interactions through molecules such as β1 and β2 integrins and CD44 with the BM stroma are of critical importance in the regulation of hematopoiesis and lymphopoiesis by retaining the progenitor cells in close proximity with the costimulatory signals and the various cytokines they produce (109). It is possible that the leptin signaling-competent environment in the WT recipient could fully compensate for the absence of leptin receptor on the transferred *db/db* BM cells with the abundant supply of cytokines and growth factors in contrast to the BM environment in the *db/db* mice, since leptin has been shown to be capable of inducing expressions of certain cytokine such as IL-12 and IL-6, etc. Moreover, this observation also implies a possibility for functional redundancy of leptin signaling with the effects of the cytokines required for lymphopoiesis, based on the evidence of the structural similarity between leptin and some cytokines produced by the BM stroma such as IL-6, IL-12, IL-15 and G-CSF, etc.

Role of leptin in innate immunity

The role of leptin in innate immunity has been demonstrated by a wide range of leptin actions on antigen-presenting cells (APCs), NK cells and neutrophils (Figure 2). Congenital leptin deficiency in human displayed dysfunctional immune function such as an increased incidence of infection-related death during childhood (110, 111) and leptin therapy has been shown to correct multiple immune abnormalities in these patients (111). Both *db/db* and *ob/ob* mice exhibit defective cell-mediated immunity and lymphoid atrophy with enhanced susceptibility to infection and injuries (112-115). Peritoneal macrophages in *ob/ob* mice display impaired phagocytic capacity which rendered them unable to clear bacterial infection (14). The findings that leptin expression can be induced rapidly by inflammatory stimuli such as LPS, IL-1 and TNF-α during the acute phase of immune response indicate a role for leptin acting as a mediator in regulating

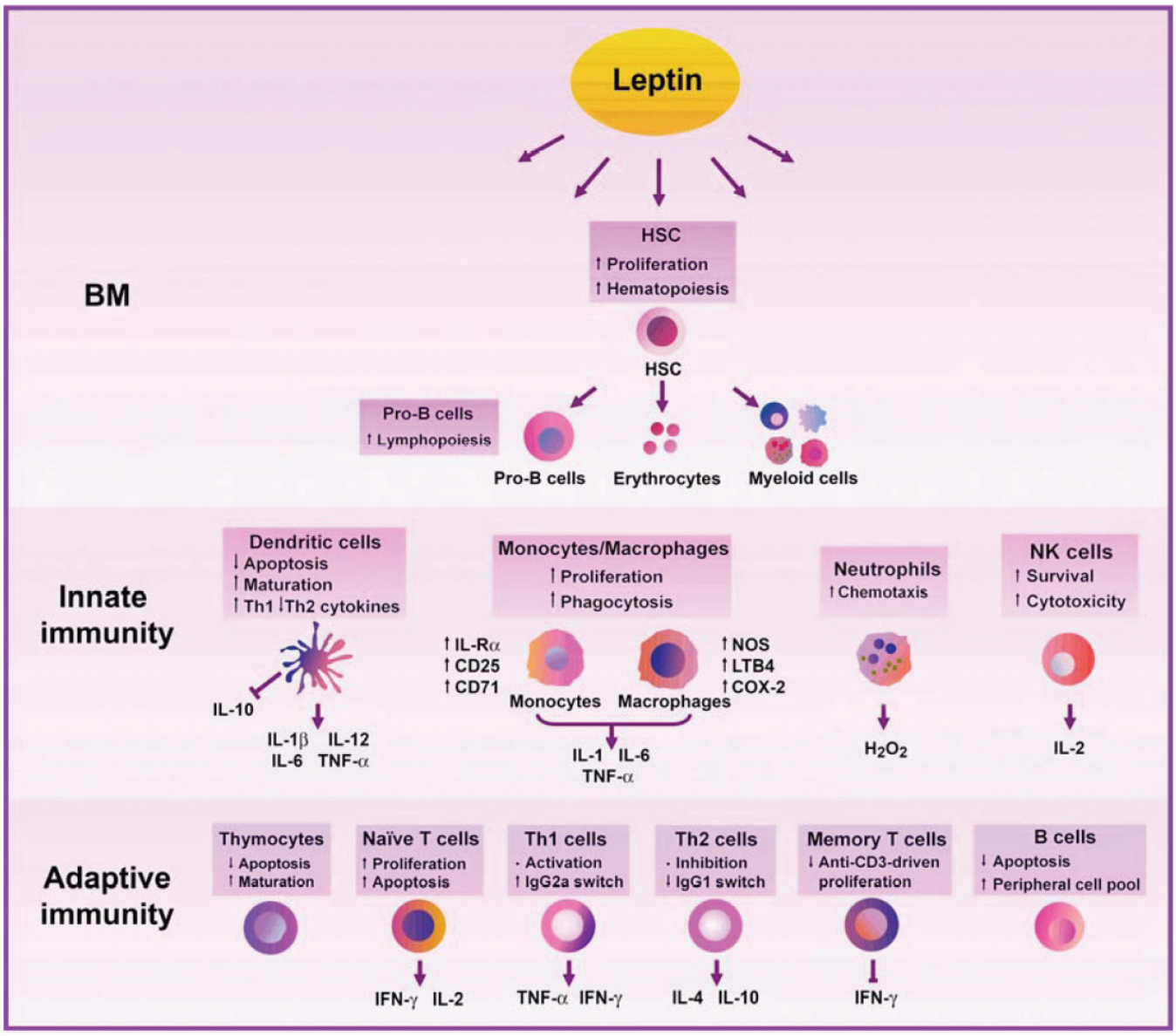

Figure 2. Effects of leptin in immunity. Leptin can stimulate the proliferation of stem cells and hematopoiesis into lymphoid, erthyroid and myeloid lineages. In B cell development, it also has a direct role for leptin in stimulating lymphopoiesis. Leptin have shown direct effects in both innate and adaptive immunity. It can stimulate DC, monocytes, macrophages, neutrophils and natural killer (NK) cells. Leptin is involved in DC maturation and survival, and can skew the cytokine balance of a Th1 profile. In monocytes and macrophages, leptin has been shown to stimulate proliferation and phagocytosis, together with production of pro-inflammatory cytokines. In macrophages, leptin can induce production of factors involved in regulating immune responses such as nitric oxide, leukotriene B4 (LTB4), cholesterol acyl-transferases-1 (ACAT-1) and cyclo-oxygenase 2 (COX-2). Leptin can induce the expression of IL-1Rα, CD25 and CD71 in monocytes and stimulate chemotaxis and release of hydrogen peroxide in neutrophils. Moreover, leptin is required for normal NK cell development and enhances their cytotoxicity. In adaptive immunity, leptin is essential for thymic homeostasis by its anti-apoptotic functions and maintenance of thymic maturation. Leptin can promote naïve T cell survival and production of IFN-γ and IL-2, and activate Th1 cells while inhibiting Th2 cells. On Th1 cells, it increases their TNF-α and IFN-γ production, and IgG_{2a} switching by B cells. In contrast, leptin exerts inhibitory effects on Th2 cells by reducing IgG_1 switching. In memory T cells leptin has been shown to inhibit anti-CD3-driven proliferation but stimulate their production of interferon-γ. As a survival factor, leptin has been shown to suppress B cell apoptosis.

inflammatory activities (14, 116). Studies by our group and others have shown that leptin signaling participates in innate immunity by promoting the maturation and survival of DC (9, 10). In the absence of leptin signaling, DC display a Th2-biased cytokine profile while exogenous leptin treatment skewed the cytokine balance of normal DC towards a Th1 profile (9, 10). The altered Th1:Th2 cytokine balance is associated with a corresponding change in the immuno-

stimulatory capacity on T cells. DC from *db/db* mouse BM or WT DC treated with a leptin receptor chimera show increased apoptosis along with defective Akt and NF-κB signaling pathways (10). Leptin also plays a role for the inflammatory response in diabetes-impaired skin repair, where treatment with leptin in the diabetic *ob/ob* mice with leptin promotes macrophage influx into the wound site (25). In monocytes and macrophages, leptin has been shown to stimulate proliferation and phagocytosis, together with production of pro-inflammatory cytokines. In macrophages, leptin can induce production of factors involved in regulating immune responses such as nitric oxide, leukotriene B4 (LTB4), cholesterol acyl-transferases-1 (ACAT-1) and cyclo-oxygenase 2 (COX-2) (116-120). In *ob/ob* mice, the increased susceptibility to LPS- and TNF-α-induced mortality correlates with significantly lower concentrations of IL-1 receptor antagonist IL-1Rα. Leptin administration can induce the expression of IL-1Rα in monocytes (115, 121). Leptin also upregulates the expression of activation markers such as CD25 and CD71 in human monocytes (116) and stimulates chemotaxis and release of hydrogen peroxide in neutrophils (11). In peripheral blood mononuclear cells, leptin induces the production of growth hormone by protein kinase C (PKC)- and nitric oxide-dependent pathways (118). Moreover, leptin signaling deficiency has been shown to impair NK cell development and function in the *db/db* mice with significantly reduced peripheral NK pool size (13). Further *in vitro* studies show that leptin enhances the cytotoxicity of NK cells by upregulating IL-2 and perforin expressions through direct activation of STAT3 (12). Apart from cytotoxicity, leptin exerts a wide range of actions by affecting proliferation, differentiation and activation in NK cells (12, 13). These data supports a pleiotropic role of leptin in maintaining immune homeostasis by regulating the survival and activity of immune cells and innate immune response.

Role of leptin in adaptive immunity

The functions of leptin in stimulating the pro-inflammatory cytokine production involved in innate immune responses can indirectly modulate the adaptive immunity. For example, the leptin-induced type I cytokines such as IL-12 and TNF-α in DC serve to license naïve $CD4^+$ T cells for Th1 priming (9). However, leptin also plays a direct role in adaptive immunity by modulating T cell-mediated immune responses, as evident by the expression of the functional leptin receptor OB-Rb in different T cell subpopulations and B lymphocytes (Figure 2). Early studies on *db/db* mice revealed that the development and maturation of both T and B cells are severely affected with reduced numbers of lymphocytes in peripheral lymphoid organs (102). The immune abnormalities in immune responses are observed in *ob/ob* and *db/db* mice, as well T cells in the *ob/ob* mice (8), indicating a protective role of leptin in enhancing T cell survival. In a recent study, treatment with pharmacologic doses of leptin in the *ob/ob* mice stimulates thymopoiesis even in the LPS-induced thymic atrophy (122). Furthermore, leptin has been shown to promote the survival of both T and B lymphocytes by suppressing Fas-mediated apoptosis, which may result from its induction of the anti-apoptotic proteins including Bcl-2 and Bcl-X_L and downregulation of Bim (10, 123, 124). Leptin also increases the production of a variety of pro-inflammatory cytokines such as IFN-γ and IL-2 in T lymphocytes (7, 14, 116, 125, 126) and modulates the immune response towards the Th1 phenotype by stimulating CD4 in mice with starvation-induced leptin deficiency (50). Thymic atrophy induced upon starvation was prevented by leptin replacement, and leptin also protected thymocyte apoptosis by maintaining thymic maturation of the double positive $CD4^+CD8^+$ T lymphocyte proliferation with activation of STAT3 and its DNA binding activity (7, 53). In Th1 cells, leptin increases their TNF-α and IFN-γ production and IgG_{2a} switching by B cells. In contrast, leptin exerts inhibitory effects on Th2 cells by reducing IgG_1 switching. Consistently, $CD4^+$ T cells from *db/db* mice showed impaired proliferative capacity (53). Notably, leptin can modulate specific aspects of T cell function with differential effects on distinct subpopulations of lymphocytes, as demonstrated by the findings that leptin can stimulate proliferation of $CD4^+CD45RA^+$ naïve T cells but inhibit anti-CD3-driven proliferation of $CD4^+CD45RO^+$ memory T cells while stimulating their production of interferon-γ (125). These studies indicate the diverse actions of leptin in regulating immune homeostasis.

Although the immunomodulatory role of leptin in immunity has become increasingly evident, the other major function of leptin as an endocrine hormone to regulate energy storage and metabolism has added complexity in ascertaining the leptin effects either in immune modulation or in metabolic functions. This requires cautious interpretations of the observed immune deficits in the *ob/ob* and *db/db* mice, as hyperglycemic and insulin resistant conditions that occur during their early adulthood can affect the immune system indirectly. However, extensive studies *in vitro* with the specific blockade of leptin signaling have provided clear evidence for the leptin effects in immune cells. Moreover, our study using young *db/db* mice at a prediabetic stage with normal glucose levels showed impaired DC maturation and survival supports a distinctive role of leptin in regulating DC development and function (10). Importantly, severe combined immunodeficient (SCID) mice transferred with leptin receptor deficient $CD4^+CD45RB^{hi}$ T cells isolated from *db/db* mice showed delayed onset of colitis and reduced IFN-γ production, demonstrating a direct role of leptin in immune modulation (127).

Role of leptin in autoimmune diseases

A growing body of evidence indicates that leptin acts as a pro-inflammatory cytokine in immune responses. Although pro-inflammatory factors are critical mediators of host defence mechanisms, these cytokines can negatively associate with the development of autoimmune diseases.

Leptin has also been shown to enhance immune reactions in autoimmune diseases that are commonly associated with inflammatory responses. The prevalence of autoimmune diseases such as RA is increased with serum leptin levels. Recent evidence indicates that leptin is involved in the dysregulated balance between Th1 and Th2 cytokines and contributes to the pathogenesis of RA (128). In contrast, leptin deficiency has a protective effect on autoimmune diseases by altering the balance of Th1:Th2 cytokine production and promoting a Th2 response, as shown in fasting RA patients exhibiting significantly improved clinical disease activity correlated with a marked reduction in serum leptin and a shift toward Th2 cytokine production (129). Consistently, less severe autoimmune arthritis has been observed in both *ob/ob* and *db/db* mice following the immunization of methylated BSA into knee joints, and the antigen-specific T cell proliferative responses are markedly decreased in *ob/ob* mice, suggesting an involvement of leptin signaling in antigen-induced arthritis (130). The immunomodulatory effects of leptin have also been linked to enhanced susceptibility to other autoimmune disease such as experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis (15, 131). Recent studies have shown that leptin is involved in the induction and progression of EAE (132, 133). The *ob/ob* mice are resistant to EAE induction with increased IL-4 and a lack of IFN-γ after myelin-specific stimulation of T cells. Interestingly, leptin replacement renders these mice to become susceptible to the disease accompanied by a shift to Th1 type cytokine pattern and reversal of IgG_1, a Th2 dependent antibody, to the Th1 dependent IgG_{2a} (134). The role of leptin in EAE is further established with leptin neutralization in WT mice which significantly improves clinical score and delayed disease progression by inhibiting T cell autoreactivity during EAE progression (132). In both experimentally-induced colitis and hepatitis models, *ob/ob* mice exhibit a dramatic reduction of colitis severity along with reduced serum levels of TNF-α and IL-18 (135). Leptin replacement converts disease resistance to susceptibility with spontaneous release of pro-inflammatory cytokines in these mice. The influence of leptin deficiency has been examined on immune-mediated renal disease and accelerated nephrotoxic nephritis in the *ob/ob* mice which were found to be strongly protected from the disease (136). Beside experimentally-induced autoimmune diseases, leptin is also involved in spontaneous autoimmune disease such as T1D in the non-obese diabetic (NOD) mice. Leptin accelerates the disease onset and progression by stimulating autoimmune destruction of β-cells and significantly increased IFN-γ production in peripheral T-cells (15). A recent study has shown that a spontaneous mutation of the leptin receptor in normally type 1 diabetes-prone NOD mice suppresses T1D development in the NOD mice by inhibiting activation of T-effector cells, demonstrating the important role of leptin signaling in the disease pathogenesis (137). Finally, lepin appears to also play a role in chronic graft-versus-host disease (cGVHD) in patients who receive hematopoietic stem cell transplantation (138), in which increased serum leptin levels are associated with the development of cGVHD. In relation to the important role of leptin in autoimmunity, women are 2-3 times higher in serum leptin levels than men adjusted for age and BMI, and are predisposed to autoimmune diseases such as multiple sclerosis, RA, and systemic lupus erythematosus. Therefore, leptin might play a part in the prevalence of autoimmune conditions in females. Recent clinical studies on autoimmune disease patients demonstrate that high serum leptin levels may either play a causal role in the disease progress or serve as a diagnostic marker for clinical application (139).

T regulatory cells (Tregs) have vital functions to suppress the activity of $CD4^+CD25^-$ T cells that mediate the autoreactive responses and have been implicated in the treatment of autoimmune diseases such as T1D. Expanded antigen-specific Tregs can be transferred to NOD mice to ameliorate or even prevent the progression of diabetes. Recently, it has been reported that the increased leptin levels in EAE associate with reduced frequency of $CD4^+CD25^+$ Tregs cells (140). While significantly increased Tregs are found in both the *ob/ob* and *db/db* mice, treatment with the leptin receptor blocker in the WT mice can also increase the percentage of Tregs. In contrast, leptin blockade was shown to delay the onset and progression of EAE which demonstrates an inverse correlation between leptin serum levels and Treg frequency.

Nevertheless, it remains to be studied whether leptin regulate Tregs directly or *via* the action of other immune cells. It also remains largely unclear how leptin might regulate the tolerogenic immunity and whether leptin modulates immune suppression by inhibiting the production of the Th2 cytokines such as transforming growth factor-beta (TGF-β). We have previously reported increased IL-10 production in the *db/db* mice-derived DC and other studies have shown increased serum levels of IL-10 and TGF-β in both *ob/ob* and *db/db* mice. Both TGF-β and IL-10 are vital factors for the positive regulation of Tregs. With the increasing focus on Tregs being evaluated as therapeutic target in treating autoimmune diseases, further understanding of the mechanisms underlying immune response and tolerance induction will benefit the development of therapeutic treatment for autoimmune diseases (105, 141-144).

Concluding remarks and future perspectives

Although increasing evidence demonstrates leptin as a pivotal mediator in host defence against infection, immune responses, and even the induction of autoimmune diseases, it remains to be established if leptin can serve as a potential therapeutic target in treating human autoimmune diseases. Administration of leptin does not efficiently alter pro-inflammatory cytokine levels and immune function in the normal or obese individual, but immune dysregulation can be reversed by leptin replacement in congenital leptin deficient individuals (111). Several studies have shown that normal mice are responsive to leptin treatment and that modulation of leptin can target autoimmune diseases in these animals.

However, a recent study showed that leptin administration selectively stimulates thymopoiesis only in the *ob/ob* mice but not in the WT control, or when there is LPS-induced thymic atrophy (122). These observations suggest that while there is an absolute requirement for a basal level of this cytokine in maintaining its functions, leptin might act a redundant signal under certain conditions. Nevertheless, further studies will be needed to test if leptin treatment can affect the pathogenesis of autoimmune diseases in humans. Obesity has long been recognized as a risk factor for tumorigenesis. Accumulating evidence suggests leptin as a potential link between obesity and cancer development. Whereas leptin serves as a pivotal factor in immune surveillance, it has been shown to act as a mitogenic agent to promote the proliferation and invasiveness of certain cancer cells (145). Therefore, further studies are required to delineate the differential roles of leptin in these complex conditions. Since leptin exhibits pro-inflammatory functions that mediate the cellular immune response, it can be utilized as an adjuvant in the development of DC vaccines. Similarly, the management of leptin levels in autoimmune states may open up new possibilities. Furthermore, the development of various antibodies against leptin or leptin receptor mutants with antagonistic properties may hold promise as a therapeutic option for autoimmune disorders or other diseases.

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