Leptin and Immunology of Obesity
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Abstract
Today, obesity is a major health problem in both developing and developed countries and investigations discovering its mechanisms are ongoing. Obesity is considered as a state of low-grade inflammation and it is believed that inflammation could be regarded as cause or consequence of obesity. Among all studied factors involved in obesity, leptin has been subject of extensive research. Leptin is a product of the ob gene and is regulated dynamically in the body. Leptin, which is mostly produced by adipocytes, has a role in satiety and is elevated in obesity. It can affect many cells in the immune system and is a target in immunological approaches. Other energy-regulating molecules are also under investigation and research in this field can help to discover new treatments for inflammatory diseases and also to reverse obesity and prevent its disastrous complications.

Keywords: Obesity, Inflammation, Leptin, Adiponectin

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**Introduction**

Obesity is a multifactorial condition, and its onset and subsequent development is a result of multiple interactions between genes and the environment (1). It is a major public health problem in developed and developing countries such as Iran (2). Obesity is associated with many medical conditions such as asthma (3, 4), sleep-disordered breathing (5, 6), iron deficiency (7) and certain nutrient deficiencies (8). The complex phenomenon of obesity is the subject of intensive research to uncover the mechanisms involved in its pathogenesis and to discover ways of reversing it. Several processes, molecules and diverse cells are involved in obesity, among which we will discuss the inflammatory process, leptin, adiponectin and ghrelin molecules, T cells, antigen presenting cells, eosinophils and Natural Killer cells.

**Inflammation and obesity**

Obesity is a state of chronic low grade systemic inflammation (9) and microarray studies have shown altered gene expression of cytokines, chemokines, complement proteins and other acute phase moieties in obese subjects (10). It has also been proposed that chronic activation of the innate immune system could be regarded as a possible risk factor in the development of the obesity and its associated inflammation (11). Therefore, inflammation is believed to be both an effect of obesity and a contributor to its progression and sequel. Many factors are involved in the inflammatory process. This review will discuss the role of microbes, food intake and cytosolic candidates.

**Microbes**

Germ-free mice are resistant to diet-induced obesity, an observation that implicates that, microbes have an adipose-inducing role. It has been shown that mycobacterium tuberculosis infects adipose tissue and adenovirus 36 infects adipocytes. Microbes may also release a product that localizes to fat tissue and they also may be the cause of the development of innate immune responses (11). Toll-like receptors (TLRs) are a group of receptors belonging to Pattern-recognition receptors (PRRs) and they recognize multiple pathogen-associated molecular patterns (PAMPs) such as fragments of bacterial cells (12). TLRs and notably TLR4 have been shown to be expressed on innate and adaptive immune cells as well as adipocytes. Thus their activation by microbes may link microbial-induced inflammation with obesity (11).

**Food**

Apart from the rare mutations in genes encoding hormones which are involved in food intake and metabolism and their receptors, the biggest contribution of food to obesity might be the consumption of total calories. Fat (saturated and trans fatty acids) and glucose can also be candidates for the rising prevalence of obesity. Fat, glucose and their products might engage cell surface receptors such as CD14/TLR4/MD2 receptor complex on macrophages and trigger pro-inflammatory signals (11). Recent findings show that TLRs and especially TLR4 mediate the link between the metabolic and immune systems by sensing lipopolysaccharides (LPS) and also endogenous lipids and free fatty acids. This process leads to expression of inflammatory mediator genes such as TNF-α, IL-1, IL-6 and iNOS (12).

Both cells of the innate immune system and adaptive immune response, notably T- or B lymphocytes and dendritic cells, express certain TLRs and respond directly to corresponding ligands in concert with TCR or BCR signals of lymphocytes. Thus fat and glucose, via TLRs, not only stimulate innate immunity, but also shape the adaptive immune response from its initiation to the development of immunological memory (11).

**Reactive Oxygen Intermediates and Reactive Nitrogen Intermediates**

 Reactive oxygen and nitrogen intermediates (ROIs and RNIs) are generated by metabolism and inflammation. Depending on the amount and duration of their production, they regulate intracellular signaling and gene expression. High levels of these mediators induce apoptosis, necrosis, cytostasis and mutagenesis. They also regulate each other’s generation.

Studies show that ROI production increases through the exposure of adipocytes to fatty acids and NO synthesis blockade in mice with...
high fat diets, increases insulin sensitivity and reduces weight at the same time. In fact, in the presence of elevated glucose, RNI and ROI production increases. In vitro and in vivo high glucose induces adipocytes, endothelial cells and vascular smooth muscle cells to synthesize more ROI. Metabolism-triggered ROI and RNI start feedback cycles that ultimately result in their increase. As an example, ROI drives cells to produce a lipid aldehyde which reduces glutathione and so induces TNF-α, interleukin 1β, interleukin 6 and chemokines. The chemokines can attract and activate macrophages to produce more ROI and RNI. These intermediates can damage DNA, causing activation of NF-κB, which in turn enhances their cellular production. High glucose along with cytokines also induces iNOS in vascular smooth muscle cells (13).

**Energy-regulating molecules and obesity**

There are factors derived from adipose tissue and neuroendocrine system, which apart from regulating food intake and metabolism, influence the immune system. These factors include adiponectin, visfatin, neuropeptide Y, and ghrelin (14) of which we discuss leptin, adiponectin and ghrelin here. (Fig 1)

**Leptin**

Leptin, a member of helical cytokine family (which includes IL-6, IL-11, IL-12, G-CSF, and has a similar structure to IL-2) is a 16-kDa nonglycosylated protein, produced by the ob gene and mostly secreted by adipocytes. Leptin is regulated dynamically which means it decreases by fasting and increases by inflammatory mediators (15). It also might be regulated by the circadian rhythm (16, 17), food intake and exercise (18). Leptin's most crucial role is its inhibitory effect on appetite (15) via a feedback mechanism signaling to the brain the size of available energy resources. Its plasma concentration correlates positively with adipose tissue mass (19). The leptin receptor, a member of the class I cytokine receptor family (20), has at least 6 isoforms and is a product of the db gene (19). While its signaling pathways involve JAK-STAT, PI 3-kinase, MAPK, AMPK, and mTOR pathway, positive (SH2B1) and negative (SOC3) regulators also control its signaling (21). The receptor expression is highest on hypothalamus, then in peripheral tissues and finally in peripheral T cells, NK cells, monocytes-macrophages, eosinophils and B cells (15, 19, 22, 23). Human T regulatory (T reg) cells, the cells critical in down-regulation of immune responses and self-tolerance (24), are also reported to constitutively express high amounts of leptin and leptin receptor and the leptin pathway can act as a negative signal for the proliferation of these cells (25). This fact may confirm the role of leptin in autoimmunity in accordance with studies investigating leptin in ankylosing spondylitis, MS and rheumatoid arthritis (14, 26, 27). It has also been proposed that leptin can play a role in the development of lung cancer by stimulating innate and adaptive immune responses and stimulating chronic inflammation (28).

**Ghrelin**

Ghrelin discovery helped to link the gastrointestinal tract and nutritional intake to the hypothalamic-pituitary unit. Ghrelin, a 28 amino acid peptide displays strong growth hormone (GH)-releasing activity. In addition to stimulating food intake and body weight gain, it also exerts diverse actions in circulation, digestion, cell proliferation and immune functions (29, 30). An example of its immune functions is blocking leptin-induced secretion of proinflammatory cytokines by human T cells (14).

**Adiponectin**

Adiponectin is an insulin-sensitizing hormone which, unlike leptin, declines with the expansion of adipose tissue observed in obesity (31). It is composed of a globular and a collagenous domain, which after synthesis, forms trimers which then oligomerize into polymers of 4 to 6 trimers (31). It has important anti-inflammatory effects in obesity. Adiponectin acts on macrophages and monocytes to inhibit proinflammatory cytokine production and to increase IL-10 and IL-1 receptor antagonist expression. It reduces the induction of the endothelial adhesion molecules ICAM1 and VCAM1 by either TNF-α or resistin (31, 32). Three adipokine receptors expressed in different tissues have
been discovered for adiponectin and obesity has been defined as a state of adiponectin resistance coupled with adiponectin decline and possibly loss of receptor population in liver and muscle (32).

**Fig 1-** Schematic representation of leptin and adiponectin in different weight states. Leptin secreted by adipose tissue and also immune cells exerts different effects on effectors and regulatory T cells. The effects of adipokines (leptin, adiponectin and other adipokines) together with other genetic and environmental factors determine the disease susceptibility to autoimmune or infectious diseases.

**Immune cells and obesity**

**T cells**

T cells are an important arm of adaptive immunity and depend on leptin for survival signals during the energy-consuming processes of lymphocyte maturation (14). Leptin is also responsible for inducing proliferation of naïve CD4+CD45RA+ T cells and inhibiting proliferation of memory CD4+CD45RO+ T cells (14, 33). Leptin also exerts antiapoptotic activity on T cells (34). On the other hand, T cells produce leptin and up-regulate the expression of leptin receptor after activation (14). It has been stated that morbidly obese women have higher counts of T cells and lymphocytes (35).

Among T cells, T reg cells play a central role in maintaining peripheral tolerance to self-antigens and regulating the immune response to non–self-antigens. Diverse T reg populations exist, of which natural and adaptive T reg cells are CD4+ CD25+ FoxP3+. IL-10-secreting regulatory T cells (IL-10 Treg cells) as another group of T cells, are induced in the periphery in response to antigenic stimulation and secrete large amounts of IL-10. Treg type 1 cells (TR1 cells) are a subgroup of IL-10 Treg cells which are CD4+ CD25+ FoxP3-. TH3 cells as another population of T reg cells, produce large amounts of TGF-β and play a crucial role in inducing and maintaining peripheral tolerance by driving the differentiation of Foxp3+ regulatory cells in the periphery (36-38).

Some rationales exist in obesity to justify the investigation of T reg cells. First, the existence of low-grade inflammation could be a sign of defective T reg number and/or function and Th1 polarity of CD4+ T cells that may indicate T reg impairment (39). Besides, white adipose
tissue is a source of ghrelin, leptin and cytokines that may have an effect on T reg cells (40). Third, T reg cells have been known to have a role in atherosclerosis, a complication of obesity (39, 41, 42). However, a preliminary study with limited number of participants did not confirm any change in the proportion of T reg cells. In this study, only FoxP3+ T reg cells have been investigated while other populations including TR1 cells have not been evaluated (39). A comprehensive study to examine the role of leptin in the control of T reg cell proliferation showed that leptin can modulate the hyporesponsiveness and proliferation of T reg cells both in vitro and in vivo. As a result, leptin neutralization combined with TCR signaling causes expansion of T reg cells, reverses anergia and hyporesponsiveness and inhibits the proliferation of effectors T cells (25). Recent studies have focused on the role of microbiota of the intestinal tract in the induction of T reg cells by certain microorganisms to prevent and alleviate inflammatory diseases (11).

**Antigen presenting cells**

Dendritic cells (DCs) are the most potent antigen presenting cells (APCs) and have important roles in the generation and regulation of immunity. According to the study of Mattioli et al, immature and mature DCs both express leptin receptor and up-regulate it by leptin treatment (15). However, leptin does not induce changes in DC phenotype while up-regulates cytokines (IL-12 in mature DCs and IL-1β, IL-6, and TNF-α in immature and mature DCs) and chemokines (MIP-1α). It also up-regulates the immunostimulatory capacity of DCs. Through the rearrangement of the microfilament system in DCs, leptin induces morphological changes typical of cells undergoing migration, activation and cell-to-cell interaction. Leptin-treated DCs were also shown to drive T cell polarization towards the Th1 phenotype (by up-regulation of IL-12 and down-regulation of IL-10) and to be protected from spontaneous and induced apoptosis(15).

In macrophages, another type of APCs, leptin upregulates secretion of proinflammatory cytokines such as TNF-α, IL-6 and IL-12. Leptin can also stimulate the proliferation of human circulating monocytes and upregulate the expression of activation markers (14). Dendritic cells and monocytes as APCs also influence T reg induction and activation. They are downregulated by T reg cells, forming a mutual relationship as well. Therefore, adipokines excreted by the fat tissue and other factors of low-grade chronic inflammation present in obesity may alter the function of APCs in obese subjects and theoretically these APCs affect T reg cell population (39). In spite of these facts, a study investigating the circulating DCs and monocytes in obese subjects in context with T cell characterization, did not find any significant alterations in the prevalence of DC subtypes or cytokine producing capacity of stimulated monocytes (39).

Furthermore, adiponectin, an adipose tissue secreted cytokine, which is reduced in obesity, inhibits the expression of TNF-α in monocytes. Thus, as reported in obese women, stimulated monocytes produce higher levels of TNF-α.

**Other cells**

Leptin directly activates eosinophils and delays their apoptosis by inhibiting proapoptotic events(23). Leptin can cause eosinophil migration and accumulation at inflammatory sites such as lung and airways by improving the adhesion of eosinophils to bronchial epithelial cells and transmigration into inflammatory sites (19). It can induce newly synthesized IL-1β, IL-6 and chemokines such as MCP-1 and IL-8 in eosinophils thus mediates Th2 immune responses (19). Leptin increase has been associated with atopic asthma, atopic dermatitis and allergic inflammatory diseases (23) possibly due to decreased immune tolerance (43). Preliminary studies of leptin in Natural killer (NK) cells have demonstrated that recombinant leptin improves proliferation and cytotoxicity of NK cells. Upregulation of NK cell functions are exerted through activating STAT3 signaling and improving gene expression of IL-2 and perforin (22). It has been shown in some studies that leptin has Chemotactic activity towards neutrophils while Inhibits neutrophil chemotaxis to classical neutrophilic chemoattractants (44) (Fig 2).
**Th1/Th2 responses and obesity**

Th1 shift in obesity has been reported in some studies (15, 33, 39, 45) but the factors responsible for this skewness are not fully understood. The increased production of pro-inflammatory and anti-inflammatory factors in white adipose tissue is a possible explanation, however some induce Th1 while others induce Th2 polarization resulting in an as of yet unknown net effect (39). In another possible interaction, leptin levels have been reported to prime Th1 response (14, 15, 28). The Th1-promoting effects of leptin have been linked to an increased susceptibility in developing experimentally induced autoimmune diseases including experimental autoimmune encephalomyelitis (EAE), type 1 diabetes mellitus (T1D) and antigen-induced arthritis (AIA) (14). Confirming this role of leptin, its gene transcription has also been induced concomitantly with the polarization toward Th1 responses in autoimmune diseases (14).

On the contrary, in a study by Wong et al, it has been suggested that leptin mediates Th2 immune responses by affecting eosinophils to secrete MCP-1 and IL-6 (19). Another study has also shown that TNF-α increase in obesity may induce IL-4 and -5 secretion from epithelial cells leading to Th2 immune skewness (43).

Studies on ghrelin have shown that this molecule, dose-dependently, inhibited proliferation of splenic T cells costimulated with anti-CD3. Moreover, ghrelin reduced Th1 (IL-2 and IFN-γ) cytokines mRNA expression in activated lymphocytes and completely inhibited Th2 cytokines (IL-4 and IL-10) mRNA expression (29). Furthermore, elevated CRP and ESR which contribute to the low-grade inflammation can cause dysregulation of the pro- versus anti-inflammatory and T helper (Th)1 versus Th2 cytokine balance (46).

**Diagnostic and therapeutic implications**

Leptin, acting as a pro-inflammatory cytokine, might be involved in the pathogenesis of inflammatory diseases and might be used as a marker of disease activity and clinical follow-up in autoimmune diseases such as ankylosing spondylitis and MS (14, 27).

Leptin neutralization can be a novel strategy to expand human peripheral T reg cells. Anti-leptin-based approaches could be employed for the immunotherapy of conditions characterized by low numbers of T reg cells (25). Leptin administration can also be employed to reverse the starvation-induced immunosuppression (47) and in the immune deviation from a Th2 to a Th1 response in the
treatment and prevention of allergic diseases (15). In vaccination protocols for infectious diseases, leptin may be used as an adjuvant to efficiently boost Th1 type responses (15). Recently, recombinant leptin has been administered intranasally by lactococcus lactis and has been shown to enhance adaptive immune response and reduce body weight gain and food intake (48). However, apart from the possibility of leptin resistance, that might confound the effectiveness of its treatment, leptin plays diverse roles in hematopoiesis, angiogenesis, bone and lipid metabolism, insulin secretion and the reproductive system, so in vivo treatments involving leptin may have unwanted and unpredicted effects (26). Animal studies have revealed that acute starvation resulting in reduced leptin amounts, delays the onset of autoimmune disease and sometimes ameliorates symptoms of inflammatory diseases (26).

**Conclusion**

Obesity may be considered as a low-grade systemic inflammatory disease, with diverse factors contributing to its pathogenesis and progression ranging from genetic to environmental. The association of obesity with many autoimmune and inflammatory diseases suggests a link between it and immunological dysregulation. Leptin as the famous signature of obesity has been studied intensively. Research in this field paves the way to discover new treatments for inflammatory diseases and also to reverse obesity and prevent its disastrous complications.

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