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Review

Restricted leptin antagonism as a therapeutic approach to treatment of autoimmune diseases

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ABSTRACT

Leptin, the adipocyte derived hormone, has a pivotal role in regulating energy homeostasis and appetite. Beyond this essential role in bodyweight control, leptin also regulates the immune responses. Leptin has pro-inflammatory effects on T cell populations, shifting the T helper balance towards a TH1 phenotype, through induction of pro-inflammatory cytokines and stimulation of macrophage and natural killer cell function. Acute starvation reduces serum leptin levels, resulting in an impaired cellular immune response. The TH1 pro-inflammatory immune response, a homeostatic response mediated by the low leptin levels, is also impaired during starvation. Leptin-deficient or leptin receptor mutant mice are protected against the development of several inflammatory or various T cell-dependent autoimmune diseases. Therefore, leptin appears to have a central role in the immune response and low leptin levels may protect against autoimmune disease. Here we review the role of leptin in the immune responses, with emphasis on autoimmune diseases. We will also discuss the application of leptin antagonist therapy for prevention and treatment of immunity related disorders.

Key words: Adipocyte hormone, Antagonism, Autoimmune disease, Immune response, Leptin

INTRODUCTION

The *ob*/*ob,* or obese, mouse is a mutant mouse suffering from a complex syndrome primarily characterised by excessive appetite; it becomes profoundly obese and suffers endocrinological disorders. In 1994, Jeffrey Friedman and colleagues found that leptin was the product encoded by the *ob* gene.[1] Leptin is a 16 kDa nonglycosylated protein hormone consisting of 167 aminoacids. The elucidation of its structure revealed that leptin is an α-helical-bundle cytokine.[2] Leptin consists of four interconnected anti-parallel α-helices and has a high similarity to other members of this large cytokine family, including growth hormone, interleukins such as interleukin-6 (IL-6), IL-11, IL-12, granulocytes colony stimulating factor (G-CSF), leukaemia inhibitory factor (LIF) and oncostatin M (OSM).[3,4]

Leptin is predominantly produced by adipocytes and its circulating level positively correlates with white

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adipose tissue mass. Administration of leptin to *ob*/*ob* mice increases basal metabolism and reduces food intake, leading to a remarkably rapid weight loss.[5-7] Leptin interacts with the leptin receptor, also known as LepR or Ob-R, which is encoded by the *db* gene in humans and has a single transmembrane-spanning domain. Ob-R has also been designated as cluster of differentiation 295 (CD_{295})[8] and belongs to the class I cytokine receptor super family.[9]

There are six isoforms of the leptin receptor (Ob-Ra–f): one long (Ob-Rb), four short (Ob-Ra, c, d and f) and one secreted (Ob-Re),[10,11] resulting from alternative mRNA splicing; they differ in the length of their intracellular tails but share identical extracellular-binding domains. Leptin circulates both as a biologically active free form and a presumably inactive, bound form associated with plasma proteins and the soluble leptin receptor isoform Ob-Re.[12] Ob-Rb is present in a number of hypothalamic nuclei. Leptin binds to the ventromedial nucleus of the hypothalamus, known as the "appetite center".[13] *db*/*db* mice have a deletion in the long isoform of the leptin receptor and are resistant to leptin.[14] The long isoform Ob-Rb has a predicted 306 amino acids intracellular domain in man and is responsible for most of the known effects of leptin through its complete intracellular tail, at which the signalling of four different pathways involving Janus kinase and signal transducer and activator of transcription (JAK–STAT), mitogen-activated protein kinase (MAPK), phosphatidyl inositol 3 kinase (PI_3K) and adenosine-monophosphate activated kinase (AMPK) can occur.[11] The short form (Ob-Ra) is much more widely expressed, often at higher levels, compared to the long form, e.g. in the choroid plexus, kidney, cells of the immune system, lung and liver.[4] The short isoforms might be involved in leptin transport through the blood-brain barrier (BBB) and in other unknown functions.

Interestingly, Ob-Rb is also expressed by endothelial cells, pancreatic β-cells, the ovary, CD_{34}^+ haematopoietic bone marrow precursors, monocytes/macrophages, and T and B cells.[4,15-21] Although an important role of leptin is to regulate bodyweight through the inhibition of food intake and stimulation of energy expenditure by increased thermogenesis, recent evidence has indicated that leptin is much more than a "fat-o-stat" sensor,[22] as suggested by the pleiotropic nature characterising most α-helical bundle cytokines.[23]

Leptin appears to be part of the complex network that coordinates immune responses to various stimuli. Its unique contribution may lie in integrating the body's energy status and thus adjusting the immune response to an appropriate energy level. Indeed, cell-mediated immunity is an energy-demanding process, and impairment of this immunity during starvation may save energy necessary for vital body functions. Such crosstalk between energy homeostasis and the immune system appears to be bi-directional.[24]

LEPTIN AND THE IMMUNE SYSTEM

The pleiotropic role of leptin in mammalian physiology is clearly shown by the complex syndrome exhibited by leptin-deficient *ob*/*ob* mice and leptin receptor-deficient *db*/*db* mice. These mice are not only obese but also have abnormalities in reproductive function, hormone levels, wound repair, bone structure and immune function.[18,25-29] Since administration of leptin in *ob*/*ob* mice normalised the immune dysfunctions, a direct role of leptin in the regulation of the immune system has been suggested.[26,30]

Ob/*ob* and *db*/*db* mice suffer from thymic atrophy and have reduced numbers of circulating lymphocytes.[31-33] The impaired T cell immunity in these mice indicates a direct effect of leptin on T lymphocytes. This was substantiated by the demonstration of functional expression of Ob-Rb on CD_4^+ as well as on CD_8^+ T cells.[34,35] Leptin concentration lowered by starvation appears to correlate with impaired immune responses in mice.[36] Although the risk of infection and death is highest when energy reserves are not sufficient, obesity, a state of energy excess, has also been associated with increased susceptibility to infections and poor wound healing.[37] The effects of leptin on adaptive immune responses have been extensively investigated in human CD_4^+ T cells. Leptin enhances proliferation of circulating blood T lymphocytes in a dose-dependent manner in *in vitro* conditions.[34,35] Addition of leptin, in physiological concentrations, to a mixed lymphocytes reaction (MLR) induces a dose-dependent increase in CD_4^+ T cell proliferation.[34]

Leptin appears to affect the T helper (TH) balance leading to a shift towards the T helper 1 (TH1)

subset. Leptin stimulates TH1 production of pro-inflammatory cytokines: IL-2, interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α) and IL-18, with resultant decreased production of the TH2 cytokines: IL-4, IL-5 and IL-10[34,35] (Figure 1). T lymphocytes from *db*/*db* mice do not demonstrate such responses, supporting the concept that this effect directly involves the leptin receptor expressed on the T lymphocytes. It was shown that leptin inhibits anti-CD_3 driven proliferation of memory T cells, while that of naive T cells was significantly enhanced.[38] Leptin increases the expression of adhesion molecules, such as intercellular adhesion molecule 1 (ICAM1, CD_{54}) and very late antigen 2 (VLA2, $CD_{49}B$), by CD_4^+ T cells, possibly through the induction of pro-inflammatory cytokines such as IFN-γ.[39]

Leptin also seems to be a regulator of natural killer (NK) cell development and activation. *Db*/*db* mice show decreased numbers of NK cells in the liver, spleen, lung and peripheral blood. In normal mice leptin administration increases the basal or induced lysis of splenocytes, but not in *db*/*db* mice.[40] *Ob*/*ob* mice are protected from T cell mediated hepatotoxicity. This effect obviously depends on the leptin-mediated

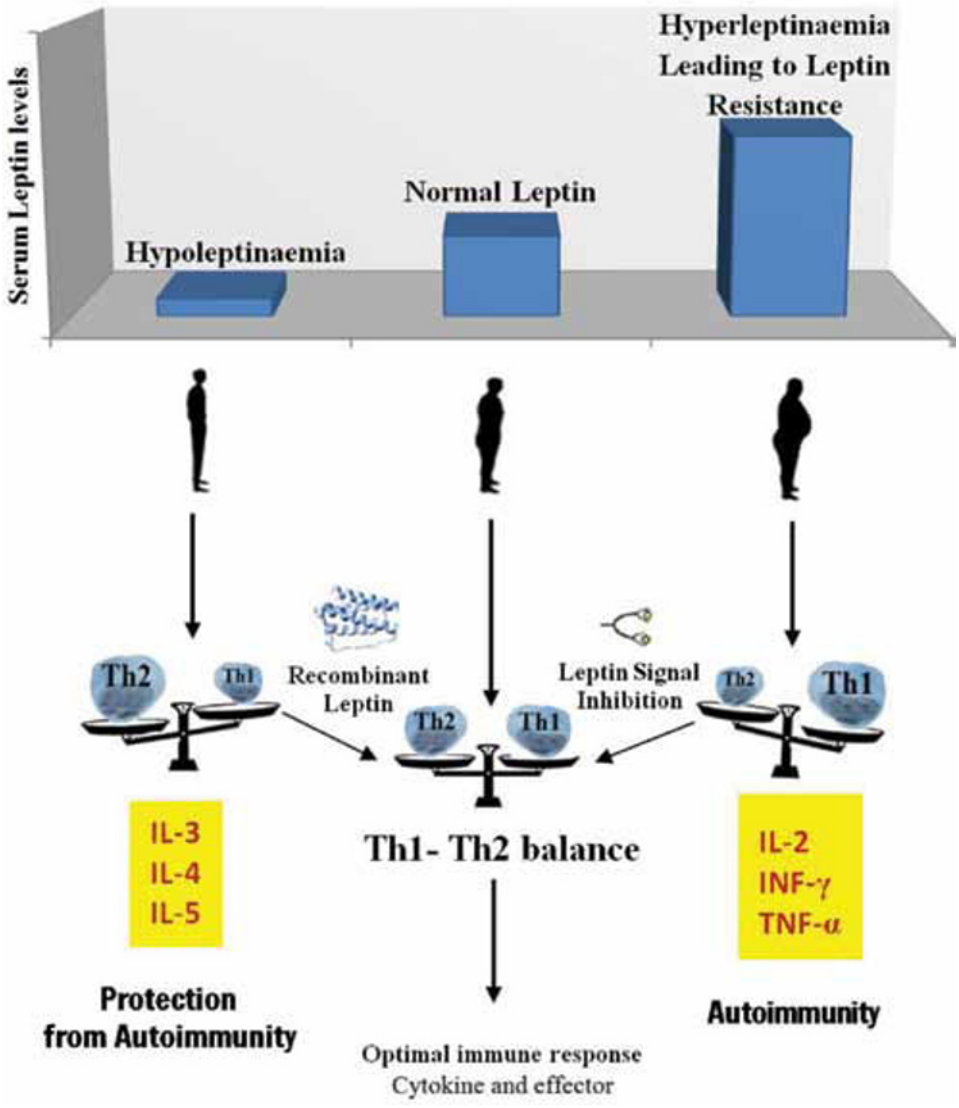

Figure 1. Relationship between Th1-Th2 balancing, autoimmune diseases and leptin. High leptin levels tend to shift toward Th1 and susceptibility to autoimmune disease, which could be reversed by leptin signal inhibition. Reduced leptin levels leads to susceptibility to infection and probably protection against autoimmune diseases.

production of the pro-inflammatory IL-18 and TNF-α cytokines.[31]

LEPTIN AND THE INFLAMMATORY RESPONSE

Leptin is one of the mediators commonly present in the neuroendocrine and immune systems.[1] In the immune system, leptin, together with C-reactive protein (CRP), IL-1 and IL-6, can act as an early acute-phase reactant, produced at high levels during inflammation, sepsis and fever, and it can also be induced by other inflammatory mediators such as TNF-α and IL-1[15,41,42] Peritoneal macrophages from *ob*/*ob* mice display a lower phagocytic activity as compared to macrophages of normal mice. Administration of leptin restored this phagocytosis deficiency.[43] Furthermore, the production of granulocyte macrophage colony stimulating factor (GM-CSF), G-CSF,[44] and the pro-inflammatory cytokines TNF-α, IL-6 and IL-12[43] by murine macrophages are enhanced after treatment with leptin. This study has demonstrated that leptin induces production of TNF-α, IL-6 and IFN-γ from resting human peripheral blood mononuclear cells (PBMCs) and enhances release of these cytokines from stimulated PBMCs.[45] It has also been shown that, at least in human neutrophils, leptin seems to mediate its effects through an indirect mechanism, probably involving the release of TNF-α from monocytes.[46] Leptin also stimulates the secretion of the IFN-γ-inducible protein in human monocytic cells, as well as in PBMCs.[47] This protein acts as a chemo-attractant for lymphocytes and monocytes,[48] recruiting activated T cells to the site of inflammation.[48,49]

All patients in a clinical trial, using recombinant leptin for weight loss, showed signs of inflammatory reaction at the site of recombinant leptin injection.[50]

LEPTIN AND AUTOIMMUNE DISEASES

Considering that congenital deficiency of leptin is associated with increased frequency of infection and related mortality,[51] it was hypothesized that a low concentration of serum leptin might contribute to increased susceptibility to infection by reducing T helper cell priming and by affecting thymic function.[26,34] On the other hand, the TH1 promoting effects of leptin have clearly been linked with an enhanced susceptibility for experimentally induced autoimmune disease including experimental autoimmune encephalomyelitis (EAE), type 1 diabetes (T1D) and antigen-induced arthritis (AIA).[52]

Leptin, as shown by evidence presented in the previous section, plays an important role in CD_4^+ T cell mediated immune responses, promoting a pro-inflammatory TH1 response. Evidence is accumulating that leptin also plays a determining role in the development of CD_4^+ T cell mediated autoimmune diseases including Crohn's disease, rheumatoid arthritis (RA), multiple sclerosis (MS) and T1D. This concept has been the subject of many researches, some of which are summarized in Tables 1A and 1B.

In experimental mouse model systems for human inflammatory bowel disease (Crohn's disease with acute and chronic colitis), leptin-deficient *ob*/*ob* mice showed a 72% reduction of colitis severity and a marked decrease of pro-inflammatory cytokines (IFN-γ, TNF-α, IL-1β, IL-18 and IL-6) in colon cell culture supernatants, compared to wild-type mice. Administration of leptin in *ob*/*ob* mice eliminates this resistance against experimentally induced colitis.[53] In another study on a murine model of inflammatory bowel disease, *Clostridium difficile* toxin A caused severe colitis but *ob*/*ob* as well as *db*/*db* mice were partially protected from toxin A-induced intestinal secretion and inflammation. Leptin administration in *ob*/*ob*, but not in *db*/*db* mice, eliminated this protection.[54]

Other data strongly indicate that leptin may be required for development of EAE, and possibly for multiple sclerosis in humans. Genetically leptin-deficient mice are resistant to induction of both active and adoptively transferred EAE. This protection is reversed by leptin administration and is associated with a switch from TH2 to TH1 type responses and IgG1 to IgG2a isotype switch. Similarly, in susceptible wild-type C57BL/6J mice, leptin worsens EAE disease by increasing IFN-γ release and IgG2a production.[55] Serum leptin levels are significantly higher in MS patients compared to sex and age matched healthy volunteers (personal unpublished data). It has also been shown that serum leptin levels in 41 women with systemic lupus erythematosus were significantly higher than in an age, sex and body mass index matched control group.[56]

Table 1A. Selected papers investigating the relationship between leptin (L) and the immune system demonstrating the influence of L on induction, maintenance and clinical manifestations of some autoimmune conditions

Year	Country/ Ref. number	Main findings	Type of study	Disease/Condition	Leptin effect on the immune system
2007	UK[75]	Plasma L was positively correlated with metabolic, inflammatory and risk factors for CVD	3640 men aged 60–79 years	CVD	L causes inflammation
2006	Israel[76]	L induces significant suppression of human hepatocellular carcinoma	Athymic nude mice		L enhances natural killer cells activity
2007	Italy[77]	L acts as a negative signal for the proliferation of human, naturally occurring $Foxp3^+CD4^+CD25^+$ regulatory T (T_{reg}) cells	Human T_{reg} cells	Autoimmune diseases	L can modulate the hyporesponsiveness and proliferation of T_{reg} cells both in vitro and *in vivo*
2005	Brazil[78]	L may be involved in some of the cellular defects observed in common variable immunodeficiency	38 patients	Common variable immunodeficiency	L causes proliferative response of lymphocytes, IL-2 and IL-4 production
2006	Czech Republic[79]	Corticosterone does not stimulate leptin production during AA	Rats	AA an experimental model of human RA	The suppression of L may be a consequence of permanent activation of NO and IL-1β.
2009	USA[80]	L induces CRP expression in human coronary artery endothelial cells via activation of the leptin receptor	Human coronary artery endothelial cells	Pro-atherogenic effects of L	Induction of CRP expression
2010	Canada[81]	Action of L on microglia is that of a modulator rather than a direct trigger of inflammation	Microglia cultures prepared from rat brain	Microglial function in inflammation	L induces production of IL-1 β, TNF-α and chemokines such as CINC-1 and MIP-2
2009	South Korea[82]	L, IL-6 and TNF-α mRNA expression of PMBCs from patients with AS were significantly higher than controls	Twenty patients with active AS and 20 healthy controls	AS	Higher L, IL-6, and TNF-α mRNA expressions in patients with AS

PBMCs: peripheral blood mononuclear cells, ITP: idiopathic thrombocytopenic purpura, CVD: cardiovascular disease, AA: adjuvant arthritis, AS: ankylosing spondylitis, RA: rheumatoid arthritis, MS: multiple sclerosis, CRP: C reactive protein.

Chronic idiopathic thrombocytopenic purpura (ITP) is an organ-specific autoimmune disease characterized by the production of antibodies against antigens on the membranes of platelets, resulting in enhanced destruction of the platelets by macrophages. Leptin enhances *in vitro* secretion of IgG anti-platelet antibodies by splenocytes and PBMCs from patients with chronic ITP. After depletion of CD_4^+ T cells from splenocytes, leptin loses this function. Further studies showed that leptin could increase platelet reactive T cells. These findings suggest that leptin may be involved in the pathogenesis of chronic ITP and thus might offer a potential target for the treatment of this disease.[57]

There are data suggesting a role for leptin in the development of RA. Injection of methylated bovine serum albumin (BSA) in the knees of mice results in the development of AIA. In contrast, *ob*/*ob* and *db*/*db* mice develop less severe arthritis as compared to wild-type mice and have decreased IL-1β and TNF-α in the knee synovial fluid and decreased serum levels of anti-methylated BSA antibodies. Furthermore, decreased antigen-specific T cell proliferative response,

Table 1B. Selected papers investigating the relationship between leptin (L) and the immune system demonstrating the influence of L on induction, maintenance and clinical manifestations of some autoimmune conditions

Year	Country/ Ref. number	Main findings	Type of study	Disease/Condition	L effect on the immune system
2005	Italy	Involvement of L and IL-6 in the action of interferon-beta in secondary, progressive MS	18 secondary progressive MS patients	MS	The effect of IFN-β on MS patients might be associated with the reduced levels of L and reduced IL-6 production by PBMCs
2006	China	L may be involved in the pathogenesis of chronic ITP	PBMCs from 18 chronic ITP patients and 14 controls	Chronic ITP	Ln enhances production of anti-platelet antibodies
2008	Poland[84]	Increased L levels in peritoneal fluid from endometriosis patients may affect local inflammatory/ immune reactions, especially infiltration of CD4+ T helper cells.	Peritoneal fluid of 46 patients and 10 control women	Endometriosis	L correlates with inflammatory cytokines (IL-1β, IL-6, IFN-γ and TNF-α)
2007	Italy[85]	ObR may be involved in the development of clinical relapses in RRMS patients	CD8+ T cells and monocytes from RRMS patients	RRMS	L-induced IL-6 production can be modulated by SOCS3 expression
2001	UK[45]	L has a direct effect on the generation of an inflammatory response.	Human PBMCs	Endotoxin stimulated and resting human PBMCs	L induces production of TNF-α, IL-6 and IFN-γ

PBMCs: peripheral blood mononuclear cells, ITP: idiopathic thrombocytopenic purpura, CVD: cardiovascular disease, AA: adjuvant arthritis, AS: ankylosing spondylitis, RA: rheumatoid arthritis, MS: multiple sclerosis, CRP: C reactive protein, RRMS: remitting relapsing multiple sclerosis.

with a lower IFN-γ and a higher IL-10 secretion, typical for a shift towards an anti-inflammatory TH2 type phenotype, has also been reported.[58] Reducing leptin levels in RA patients by fasting ameliorates the clinical signs of the disease.[39] Leptin antagonism has therefore been suggested for prevention of developing RA in people who are genetically susceptible to RA and other autoimmune diseases.

In the non-obese diabetic (NOD) mouse, an animal model for type 1 diabetes (an autoimmune disease in which the pancreatic β-cells are destroyed by inflammatory processes), an increased serum level of leptin precedes diabetes in susceptible females, while injection of leptin accelerates the autoimmune destruction of the pancreatic β-cells and increases the IFN-γ production in peripheral T cells. These effects indicate that leptin promotes the development of type 1 diabetes through TH1 responses.[59]

In MS and RA, 60 to 75% of the patients are female, and in other autoimmune diseases (thyroiditis, scleroderma, lupus erythematosus, Sjögren's disease), 85% or more of the patients are female. This is corroborant that autoimmune diseases affect women more than men.[60] This gender effect may, at least in part, reflect the higher average leptin concentrations in women.[61]

Autoimmune diseases show an increasing incidence in industrialised countries compared to less developed countries. Some researchers now believe that leptin helps to determine the balance between predisposition to infections and predisposition to autoimmune diseases. This could help explain why higher circulating leptin levels predispose to autoimmune diseases and lower circulating leptin levels to infection.[22] Based on the evidence regarding relationship between leptin and autoimmune diseases, leptin antagonism has been

proposed as an immunotherapeutic approach for the treatment of some autoimmune disorders and even in a wider range as an effective immunosuppresant.[62]

LEPTIN SIGNAL INHIBITION STRATEGIES

There are different approaches for designing antagonists. Blocking common important signal pathways, such as JAK-STAT, may result in detrimental effects. So far there is no commercially available leptin antagonist that can be used for clinical studies. The recent development of leptin mutants with antagonistic properties and other proteins that block leptin activity opens up new possibilities for their use in research and, eventually, therapy.[63]

Using site-directed mutagenesis for single amino acid residues in human leptin, which are critical for receptor binding and biological activity, has resulted in mutants with antagonistic properties that are able to interfere with the negative feedback control of endogenous leptin.[64] Binding site II in the leptin molecule is composed of residues at the surface of helices A and C. Mutations in this site impair binding to CRH2 in the leptin receptor but have only a limited effect on signalling. Some leptin mutants behave as potent leptin antagonists both *in vitro* and *in vivo*.[65] Mutations in binding site III of leptin interfere with the hexamerisation process and thus with receptor activation. A S120A/T121A binding site III leptin mutant still binds to the receptor but is unable to activate the receptor. It therefore acts as a competitive inhibitor of leptin receptor signalling.[65] Given the very short half-life of leptin in circulation (minutes), blocking effects of a leptin antagonist *in vivo* can only be observed when its half-life is extended (hours). To increase the circulation lifetime of the S120A/T121A leptin antagonist, PEGylation of this mutant has been suggested.[66]

Local delivery of leptin antagonists offers an alternative possibility. Several autoimmune diseases are limited to a specific tissue, for example, Crohn's disease to the intestine. In this particular case, local delivery of a leptin antagonist can be envisaged using a Lactococcus or Lactobacillus delivery system, as has been demonstrated using recombinant IL-10 secreting *Lactococcus lactis.*[67,68]

One of the most extensively studied and applied approaches, when it comes to antagonising cytokines, is the design of neutralizing antibodies, since they combine high binding affinity and specificity. The development of various antibodies against leptin or leptin receptor mutants with antagonistic properties may hold promise as a therapeutic option for autoimmune and/or other disorders.[69] A number of studies have described the use of anti-leptin or anti-leptin receptor antibodies for detection and quantification purposes.[70-73] A monoclonal antibody against human leptin receptor with antagonistic effect has been described. *In vitro* studies demonstrated that this mAb is able to inhibit leptin-induced TNF-α production from human monocytes and anti-CD_3 mAb induced proliferation of human T cells in PBMC culture.[74]

A critical issue concerning leptin is its pleiotropic nature, as discussed earlier. Any attempt to block the leptin signalling *in vivo* should be carefully planned as it may cause undesirable effects. The biggest advantage of recombinant antibody (rAb) technology is that rAb can be subjected to genetic manipulation (e.g. humanization, conjugation with other molecules, etc.) and more importantly can produce molecules with affinity for different epitopes which bind simultaneously to at least two different molecules. Therefore, they can block a specific molecule (such as the leptin receptor) on a specific target tissue, leaving other actions of the leptin receptor unaffected.

CONCLUDING REMARKS

The hypothalamic leptin/leptin receptor system is a determining component of the control of energy homeostasis and bodyweight. Leptin has also emerged as a pleiotropic cytokine with important effects in several peripheral tissues. However, as early leptin research has primarily been focussed on the effect of leptin on bodyweight regulation, little attention has been given to the development of leptin antagonists specifically designed for peripheral effects. Adequate nutrition is a prerequisite for generating appropriate immune responses against pathogens. Accordingly, sufficient energy stores may be one of the factors required for long-term, detrimental immune reactions, as observed in autoimmune diseases. Serum leptin is important for T cell proliferation and there is an autocrine loop of leptin to maintain T cell proliferation.[86,87]

The link between leptin and immune processes may therefore offer a new therapeutic strategy against autoimmune diseases. For this purpose there is a clear need for leptin antagonists. The monoclonal antibody against the human leptin receptor identified by our group and blocks leptin signalling, probably constitutes a promising tool for designing a tissue specific leptin antagonist. It has been shown that this antibody inhibits the pro-inflammatory activity of leptin by its ability to block peripheral immune actions of leptin and leptin-induced induction of TNF-α by human monocytes and T cell proliferation.[74]

Recent studies on the effects of leptin on the immune system suggests its decisive role in autoimmune processes. Tissue specific leptin antagonists may therefore have therapeutic value in different conditions related to the immune system including autoimmune disease, transplantation, selective immune suppression, etc.

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