Leptin as a metabolic link to multiple sclerosis

Giuseppe Matarese, Pietro Biagio Carrieri, Silvana Montella, Veronica De Rosa and Antonio La Cava

Abstract | Clinical and experimental data, together with epidemiological studies, have suggested that the pathogenesis of multiple sclerosis (MS) might involve factors that link the immune system with metabolic status. Moreover, recent research has shown that leptin, the adipocyte-derived hormone that controls food intake and metabolism, can promote experimental autoimmune encephalomyelitis, an animal model of MS. In patients with MS, the association of leptin with disease activity has been dissected at the molecular level, providing new mechanistic explanations for the role of this hormone in MS. Here, we review the intricate relationship between leptin and other metabolic modulators within a framework that incorporates the latest advances linking the CNS, immune tolerance and metabolic status. We also consider the translational implications of these new findings for improved management of MS.

Matarese, G. *et al. Nat. Rev. Neurol.* **6**, 455–461 (2010); published online 6 July 2010; doi:10.1038/nrneurol.2010.89

Introduction

Leptin is an adipocyte-derived hormone, a major role of which is to monitor fat stores in the body and, thereby, regulate energy expenditure via the neuroendocrine system.[1] In addition, leptin has been shown to influence immune function.[2] Leptin has characteristics of an early acute-phase reactant—similar to C-reactive protein and interleukin (IL)-1—and can be induced by inflammatory mediators such as tumor necrosis factor (TNF), IL-1 and IL-6. Over the past 10 years, a substantial body of evidence has implicated leptin as one of the main mediators that link nutritional status and metabolism to the immune response, and leptin and hypothalamic mediators (as well as other adipose tissue-derived cytokines) seem to have key roles in these processes.[2]

From an epidemiological standpoint, the rising frequency of autoimmune disorders and chronic inflammation in affluent countries and the Western world has prompted an increasing interest in finding new links between metabolism and autoimmune disease susceptibility.[3,4] Multiple sclerosis (MS) is one of the most common autoimmune disorders, but its etiology is poorly understood.[5] A link between metabolism and MS pathogenesis has been hypothesized, particularly in view of the finding that proinflammatory mediators such as leptin maintain microenvironmental conditions that promote loss of immune self-tolerance.[6] In this Review, we discuss this hypothesis, and consider the possibility that metabolic or nutritional interventions might directly influence the pathogenesis and outcome of MS. To set the scene, we begin with a general introduction to the roles of leptin in the immune system.

Competing interests
The authors declare no competing interests.

Leptin and the immune system

Molecular effects of leptin on T cells

The actions of leptin are mediated through the leptin receptor (LEPR; Box 1). Leptin amplifies $CD4^+$ T-cell responses, and human $CD4^+$ T lymphocytes express *LEPR* messenger RNA (mRNA).[2] The long form of LEPR, LEPRb, is expressed on both $CD4^+$ and $CD8^+$ human T cells, and was found to be expressed on regulatory T (T_{REG}) cells.[7] Human monocytes, which can be activated by high-dose leptin, also express LEPRb. In addition, human natural killer (NK) cells show constitutive expression of both the long and short LEPR isoforms.[2]

As observed with other members of the class I cytokine receptor family, the binding of leptin to LEPRb on lymphoid cells activates JAK (Janus kinase) and STAT (signal transducer and activator of transcription) proteins (Figure 1).[8,9] The cytoplasmic tail of all four membrane-bound LEPRs contains a box 1 motif, which is strongly conserved within most members of this receptor family, whereas a box 2 motif is found only in the long isoform. These two domains are involved in the interaction with and activation of JAK2 tyrosine kinase, which phosphorylates and activates members of the STAT family such as STAT1, STAT5 and STAT3. Activated JAK2 then phosphorylates tyrosine residues in the intracellular domain of LEPRb, thereby providing binding motifs for the SH protein tyrosine phosphatase-2 (SHP-2) and STAT proteins (Figure 1).[2,8,9] After tyrosine phosphorylation in response to JAK activation, the STAT proteins translocate to the nucleus, where they activate gene transcription. The STAT3-dependent target genes include *FOS*, *JUN* and suppressor of cytokine signaling 3 (*SOCS3*). Both *in vitro* and *in vivo*, LEPRb can also stimulate SHP-2-dependent ERK1/2 (extracellular signal-regulated kinases 1 and 2) activation, tyrosine phosphorylation of insulin receptor

Laboratorio di Immunologia, Istituto di Endocrinologia e Oncologia Sperimentale, Consiglio Nazionale delle Ricerche (IEOS-CNR) (**G. Matarese**, **V. De Rosa**), Dipartimento di Scienze Neurologiche, Università di Napoli "Federico II" (**P. Biagio Carrieri**, **S. Montella**), Via S. Pansini 5, 80131 Napoli, Italy. Department of Medicine, University of California Los Angeles, 1000 Veteran Avenue, Los Angeles, CA 90095, USA (**A. La Cava**).

Correspondence to: G. Matarese gmatarese@napoli.com

Key points

- Leptin is an adipocyte-derived hormone that is secreted proportionally to adipose tissue mass and inhibits food intake
- Leptin links the immune response to metabolism and nutritional status
- Leptin promotes proinflammatory immune responses and inhibits the proliferation of anti-inflammatory regulatory T cells
- Orexigenic mediators antagonize the anorexigenic and proinflammatory effects of leptin in experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS)
- Drugs that affect metabolism are effective at reducing the proinflammatory effects of leptin in EAE
- Given the beneficial effects of leptin blockade on EAE outcome, leptin could represent an attractive target to reduce autoimmune inflammation in MS

Box 1 | The leptin receptor

The leptin receptor (LEPR) is a member of the class I cytokine receptor family, which includes the receptors for interleukin 6, leukemia inhibitory factor, granulocyte colony-stimulating factor and gp120.[2] *LEPR* mRNA is alternatively spliced, giving rise to six different receptor forms known as LEPRa, LEPRb, LEPRc, LEPRd, LEPRe and LEPRf. The various LEPR molecules have cytoplasmic domains of differing lengths: LEPRb, also known as the long receptor isoform, has 302 cytoplasmic residues, which include activation and signal transduction motifs. The other membrane-bound forms (LEPRa, LEPRc, LEPRd and LEPRf) have cytoplasmic domains consisting of 34 amino acid residues, and the soluble form (LEPRe) lacks some or all of the transmembrane and cytoplasmic motifs.[2] The short forms of the LEPR are ubiquitously expressed in several tissues, where they presumably mediate leptin transport and degradation. LEPRb is primarily expressed, at high levels, in the hypothalamus, especially in the arcuate, dorsomedial, ventromedial and lateral hypothalamic nuclei, which secrete neuropeptides and neurotransmitters involved in the regulation of appetite and body weight. LEPRb is also expressed in fetal liver, jejunal epithelium, pancreatic β cells, ovarian follicular cells, vascular endothelial cells, $CD34^+$ hematopoietic bone marrow precursors, and T lymphocytes.[2]

substrate 1 (IRS-1), and phosphatidylinositol 3-kinase (PI3K) activity (Figure 1).[2,8,9]

In $CD4^+CD25^-$ effector T cells, leptin induces STAT3 phosphorylation, but stimulation of $CD4^+CD25^+$ T_{REG} cells with anti-CD3/CD28—a classic T-cell receptor (TCR) polyclonal stimulation—is not associated with induction of phosphorylation of STAT3, confirming their hyporesponsive (anergic) state.[7] SOCS3, a negative regulator of cytokine signaling, is activated by leptin neutralization in $CD4^+CD25^+$ T cells, in which stimulation with an anti-CD3/CD28 monoclonal antibody induces phosphorylation of ERK1/2 and cell proliferation, suggesting reversal of their anergic state on leptin neutralization.[7] In the same subset of T cells, levels of cyclin-dependent kinase inhibitor p27 ($p27^{Kip1}$), a molecule that is involved in the control of the cell cycle and T-cell anergy, remain elevated during anti-CD3/CD28-mediated stimulation. Leptin neutralization induces degradation of $p27^{Kip1}$, which might partially explain the reversal of anergy and subsequent proliferation.[7]

The role of leptin in immunity

Leptin is an acute-phase reactant, the secretion of which increases during bacterial infection and systemic inflammation, as well as after stimulation with lipopolysaccaride (LPS), TNF, IL-1 and IL-6. In cells of the monocyte–macrophage lineage, leptin promotes activation of phagocytosis and secretion of proinflammatory cytokines and leukotriene B_4. In human polymorphonuclear neutrophils, which express LEPRb, leptin induces the release of oxygen radicals (superoxide and hydrogen peroxide) and promotes chemotaxis.[2]

In the mixed lymphocyte reaction—an assay in which $CD4^+$ lymphocytes from one individual are cultured together with antigen-presenting cells from another individual mismatched for the human leukocyte antigen molecules—the addition of physiological concentrations of leptin induced a marked dose-dependent increase in $CD4^+$ T lymphocyte proliferative responses.[2] Leptin exerts differential effects, however, on proliferative responses and cytokine release in human naive ($CD45RA^+$) and memory ($CD45RO^+$) $CD4^+$ T cells, both of which express *LEPRb* mRNA.[2] In the presence of leptin, naive T cells exhibit increased proliferation and IL-2 production. By contrast, leptin has a minimal effect on the proliferation of memory T cells, in which it promotes increased secretion of interferon (IFN)-γ and decreased secretion of IL-4. These findings suggest a bias of leptin towards inducing proinflammatory T helper 1 (T_H1) responses, as IFN-γ is the prototypical T_H1 cytokine.[2]

These observations indicate that leptin could represent a key mediator in the link between immune responses and metabolism. Indeed, during nutritional deprivation, which is associated with low leptin levels, replacement of this hormone restored the otherwise reduced inflammatory delayed-type hypersensitivity responses both in mice and in healthy women participating in a double-blind placebo-controlled study involving acute 72 h starvation in the presence or absence of exogenous leptin administration.[10,11] Our group has produced similar findings with recombinant leptin treatment in low-body-weight women with hypothalamic amenorrhea (G. Matarese *et al.*, unpublished work).

Leptin and autoimmune disease

Animal models of MS

Leptin-deficient (*ob/ob*) mice show reduced production of IL-2, IFN-γ, IL-18 and TNF, and increased production of IL-4 and IL-10, in lymphocytes following antigenic stimulation.[2] These mice have several immune abnormalities, including impaired cell-mediated immunity, abnormal $CD4^+$ T-cell function, and thymic atrophy (which mainly affects the cortex of the thymus, where the majority of $CD4^+CD8^+$ immature T cells reside). Leptin replacement restores normal thymic function by increasing the number of $CD4^+CD8^+$ T cells, and by reducing their rate of apoptosis.[12]

Importantly, *ob/ob* mice are resistant to the induction of both actively and passively induced experimental autoimmune encephalomyelitis (EAE), the most commonly studied animal model of MS.[13] Leptin administration converts EAE resistance into susceptibility by restoring T-cell proliferation in response to myelin antigens and by shifting the predominant T_H2-type responses of the

ob/ob mice towards T_H1-type responses. Leptin administration in EAE-susceptible wild-type mice worsens EAE by increasing the secretion of proinflammatory cytokines and by favoring the production of myelin-specific autoantibodies. Taken together, these data suggest that leptin contributes to the generation of microenvironmental conditions that promote the loss of tolerance mechanisms directed towards self-reactive T cells.[13]

The onset of neurological symptoms following EAE induction is typically preceded by a reduction in food intake and body weight, and we have provided evidence that a marked surge in serum leptin levels, starting after immunization with myelin antigens, anticipates the onset of the acute phase of EAE.[11] The systemic elevation of leptin probably accounts for the inhibition of food intake and loss of body weight observed in mice with EAE, and directly correlates with clinical disease score and EAE susceptibility.

Acute and chronic inflammatory conditions induced by the administration of IL-1, TNF and LPS are associated with increased leptin levels, anorexia, and loss of lean body mass, indicating that leptin levels could link anorexia and acute inflammation.[14] Starvation reduces serum leptin and impairs delayed-type hypersensitivity responses to antigens. In female SJL/J mice—a strain that is particularly susceptible to EAE—starvation for 48 h during priming with the encephalitogenic proteolipid protein $(PLP)_{139–151}$ peptide reduced disease severity, impaired antigen-specific T-cell proliferation and IFN-γ production, and led to increased IL-4 secretion.[11] The effects of starvation could be reversed by *in vitro* and *in vivo* administration of recombinant leptin, so these data support the hypothesis that leptin, together with other proinflammatory cytokines, can directly influence demyelinating disease by favoring and sustaining T_H1 responses in the phases that precede disease onset.[11] Notably, the increase in serum leptin observed after induction of inflammation during EAE was accompanied by *in situ* production of leptin by pathogenic T cells and macrophages in lymph nodes and in acute demyelinating lesions in the brain and spinal cord of the immunized mice.

Gene microarray analysis of T_H1 lymphocytes and active human MS lesions has revealed elevated transcription of genes of the neuroimmunoendocrine axis, including the leptin gene (known as *LEP* or *OB*), the transcript of which was abundant in T_H1 cells, which are commonly involved in T-cell-mediated autoimmune diseases such as EAE.[15] In addition, anti-LEPR antibodies, as well as anti-leptin antibodies, seem to reduce the *in vitro* proliferative response of myelin antigen-specific T lymphocytes, possibly by interfering with an autocrine loop that contributes to the expansion and survival of T_H1 cells.[7] *In vivo,* treatment of EAE mice with anti-leptin antibodies or a soluble LEPR–Fc fusion protein reduced EAE onset and severity, and mortality.[16,17]

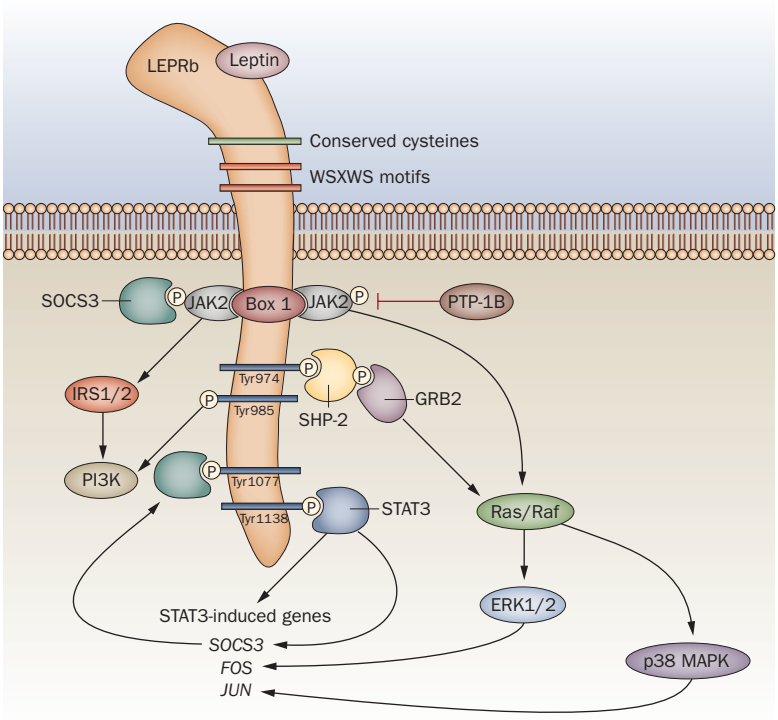

Figure 1 | Intracellular leptin signaling in immune cells. After leptin binding to LEPRb, JAK2 becomes activated by autophosphorylation or cross-phosphorylation, and phosphorylates tyrosine residues in the receptor's cytoplasmic domain. Four of the phosphorylated residues function as docking sites for cytoplasmic adaptors for STAT factors, particularly STAT3. STAT3 dimerizes and translocates to the nucleus, where it induces expression of *SOCS3* and other genes. SOCS3 participates in a feedback loop that inhibits leptin signaling by binding to phosphorylated tyrosines. SHP-2 is recruited to Tyr985 and Tyr974 and activates ERK1/2 and p38 MAPK pathways through the adaptor protein GRB2, ultimately inducing *FOS* and *JUN* expression. PTP-1B is localized on the surface of the endoplasmic reticulum, and is involved in negative regulation of LEPRb signaling through dephosphorylation of JAK2 after internalization of the LEPRb complex. JAK2 can also induce phosphorylation of the IRS1 and 2 proteins, which are responsible for PI3K activation. Abbreviations: ERK1/2, extracellular-signal regulated kinases 1 and 2; GRB2, growth factor receptor-bound protein 2; IRS, insulin receptor substrate; JAK2, Janus kinase 2; LEPRb, leptin receptor, long form; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol-3-kinase; PTP-1B, tyrosine-protein phosphatase non-receptor type 1; SHP-2, SHP protein tyrosine phosphatase-2; SOCS3, suppressor of cytokine signaling 3; STAT, signal transducer and activator of transcription.

Leptin and obesity in human autoimmunity

In addition to multiple neuroendocrine and metabolic impairments, leptin-deficient (*OB/OB*) individuals display several immune abnormalities, including an impaired cellular immune response, abnormal cytokine release (lack of IFN-γ secretion, reduced IL-4 and IL-10 levels, and increased TGF-β levels), a reduction in circulating $CD4^+$ T cells (especially naive cells), and impaired T-cell proliferation. Administration of recombinant leptin reverses these immune abnormalities and restores the $CD4^+$:$CD8^+$ T-cell ratio, T-cell proliferative responses, and cytokine production.[18]

Hyperleptinemia is associated with proinflammatory conditions and autoimmunity in humans: increased peripheral levels of leptin have been observed in patients with rheumatoid arthritis, Behcet syndrome, pelvic endometriosis and type 1 diabetes.[19] In patients with MS, leptin levels are increased in both the serum and the cerebrospinal fluid (CSF), correlating with IFN-γ production in

Table 1 | Opposing effects of anorexigenic and orexigenic mediators

Reference	Mediator	Nutritional function	Immune function	Effect on EAE
Matarese *et al.* (2001)[13]	Leptin	Inhibition of food intake (anorexigenic)	Proinflammatory	Promotion
Theil *et al.* (2009)[26] Bedoui *et al.* (2003)[27] Malfitano *et al.* (2006)[28]	Ghrelin Neuropeptide Y Cannabinoids	Stimulation of food intake (orexigenic)	Anti-inflammatory	Inhibition

Abbreviation: EAE, experimental autoimmune encephalomyelitis.

the CSF.[16] Moreover, T-cell lines that are reactive against human myelin basic protein (hMBP) produce immunoreactive leptin and upregulate the expression of LEPR after activation with hMBP. Treatment *in vitro* with anti-leptin or anti-LEPR neutralizing antibodies inhibits proliferation of these cells in response to hMBP, as well as the production of proinflammatory cytokines in peripheral blood mononuclear cells from patients with MS.[16]

At the molecular level, the expression of LEPR is higher in $CD8^+$ T cells and monocytes from patients with MS who are in relapse than in those who are in remission (or healthy controls). Relapsing patients display high levels of phospho-STAT3 (P-STAT3) and low levels of SOCS3 expression. Administration of leptin upregulates P-STAT3 only in monocytes from relapsing patients, suggesting that LEPR might be involved in clinical relapses in patients with MS.[20]

A recent report has shown that obesity at 18 years of age is associated with a greater than twofold increased risk of developing MS.[21] After adjusting for body size at 20 years of age, having a large body size at the age of 5 or 10 years was not associated with an increased risk of MS, whereas a large body size at 20 years was associated with a 96% increased risk, suggesting that the risk of developing MS is linked to obesity during adolescence. The mechanisms underlying this relationship remain unexplained, but we can infer from these data that prevention of obesity in adolescents is likely to reduce the probability of developing MS.[21] The pathogenesis and clinical progression of other autoimmune disorders, particularly joint degenerative inflammatory diseases such as rheumatoid arthritis and osteoarthritis, have been closely linked to obesity,[22,23] further reinforcing the idea that obesity is one of the factors that underlies the high frequency of autoimmune disorders observed in developed countries.[6]

Leptin and immune tolerance

A link between leptin and immune tolerance has recently been identified. In particular, both *in vitro* and *in vivo*, leptin can affect the generation, responsiveness and proliferation of T_{REG} cells, a key subset of T cells that is involved in the control of peripheral tolerance.[7,24] Freshly isolated human T_{REG} cells express high amounts of LEPR and produce substantial amounts of leptin, thereby generating an autocrine loop that inhibits the expansion of the T_{REG} cells.[7] Leptin neutralization blocks proliferation of effector $CD4^+CD25^-$ T cells but promotes the expansion of functional T_{REG} cells. These opposing effects of leptin blockade on the $CD4^+CD25^-$ T cells and T_{REG} cells are associated with differential expression of intracellular leptin and cell surface LEPR in the two cell subsets.[7]

Interestingly, in patients with relapsing–remitting MS, an inverse correlation between serum leptin levels and circulating T_{REG} cells is observed. Moreover, treatment of wild-type mice with the LEPR–Fc fusion protein leads to increased percentages of T_{REG} cells, as well as an ameliorated clinical course and delayed progression of EAE. Also, chronic leptin and leptin receptor deficiency in mice is characterized by increased percentages, absolute numbers and suppressive function of T_{REG} cells, which return to levels comparable to those in wild-type mice after leptin replacement.[7,16]

The fact that leptin can act as a negative signal for the proliferation of T_{REG} cells suggests new possibilities for leptin-based approaches in the immunotherapy of conditions characterized by low numbers of these cells. Leptin might act as an endogenous 'sensing' factor linking the environment (availability of nutrients) to circulating T_{REG} cell numbers. These effects could be relevant in the wider context of nutritional deprivation, which is thought to be a factor that increases susceptibility to infection, and is associated with the amelioration of clinical manifestations of autoimmunity.[6]

Metabolic status and immune function

Molecules that interact functionally with leptin can modulate immune function in various ways depending on the metabolic status. Mediators with orexigenic (appetite-stimulating) activity, such as ghrelin, neuropeptide Y (NPY) and endocannabinoids, have opposite effects from leptin not only on neurohypothalamic control of food intake, but also on the peripheral immune response and in autoimmune diseases such as MS (Table 1, Figure 2).[25–28] For example, ghrelin blocks leptin-induced secretion of proinflammatory cytokines in human T cells, and suppresses EAE.[25,26] NPY and endocannabinoids improve the EAE clinical score, ameliorate spasticity, and slow the progression of EAE.[27,28] Recent studies have shown that the proinflammatory anorexigenic functions of leptin are antagonized by anti-inflammatory orexigenic mediators, and this link might help to explain the importance of nutrient availability in relation to immune response.[25–28] Retrospective epidemiological analyses on the clinical history of patients with MS have shown that >60% of individuals exhibit reductions in body weight before the clinical onset of MS or relapse, suggesting that intrathecal secretion of factors with anorexigenic effects such as leptin occurs in the early phases of MS

(G. Matarese *et al.*, unpublished work). In support of this hypothesis, high levels of leptin are present in the CSF of naive-to-treatment MS patients at diagnosis.[16]

Calorie restriction: a new approach in MS?

A study by Piccio *et al.* has shown that calorie restriction (CR) can significantly increase survival rates and lifespan in experimental animal models of inflammation.[29] CR induces multiple metabolic and physiological modifications, including anti-inflammatory, antioxidant and neuroprotective effects, all of which could be beneficial in MS.[30]

CR also alters the course of EAE. In the Piccio *et al.* study, EAE was induced in mice after 5 weeks of CR or *ad libitum* feeding with a regular diet or a high-fat, high-calorie diet.[29] The clinical, histological and immunological outcomes indicated that CR ameliorates clinical EAE and reduces inflammation, demyelination and axon injury, but does not suppress immune functions. CR was associated with increased plasma levels of corticosterone and adiponectin, and with reduced concentrations of IL-6 and leptin. In further studies, calorie intake was restricted by 33% or 66% in a monophasic Lewis rat model, and EAE induction was shown to be totally inhibited in the latter but not the former group. The rats subjected to 66% CR exhibited depressed immune function, with fewer T cells in lymphoid organs, and impaired T-cell proliferation and cytokine production.[31,32]

CR could benefit EAE through multiple metabolic and cytokine or adipokine changes that ultimately lead to a reduced inflammatory response. For example, CR increases plasma levels of corticosterone and adiponectin, both of which are anti-inflammatory, and decreases plasma levels of leptin and IL-6, which are proinflammatory. Other possible mechanisms include CR-associated increases in levels of ghrelin, NPY and endocannabinoids, all of which can dampen EAE.[4,30]

Environmental factors are believed to have an important role in the pathogenesis of MS. This condition is particularly prevalent in the Western world, where high intake of saturated fats of animal origin is common. Despite speculation that diet might alter the course of MS, only a few randomized controlled studies of dietary alterations in MS have been published, none of which have involved CR. Studies conducted in the 1980s that attempted to use gluten-free or low-fat diets to manage MS failed to produce conclusive results and did not raise appreciable interest within the scientific community.[33,34] This lack of enthusiasm might be partly ascribed to the fact that the molecular mechanisms linking nutrition, metabolism and immunity had not been adequately explored at this time. In addition, the approaches that were employed focused mainly on specific aspects of the diet (reduction of gluten or saturated fatty acids), which, in contrast to chronic CR, might not sufficiently influence the overall inflammatory immune response.[30]

Despite the lack of success to date, dietary intervention remains an attractive approach in MS. CR associated with adequate nutrition—which could be safely accomplished through proper monitoring—could provide additional benefits such as improved insulin sensitivity, lower LDL and cholesterol levels and blood pressure and, importantly, reduced inflammation.

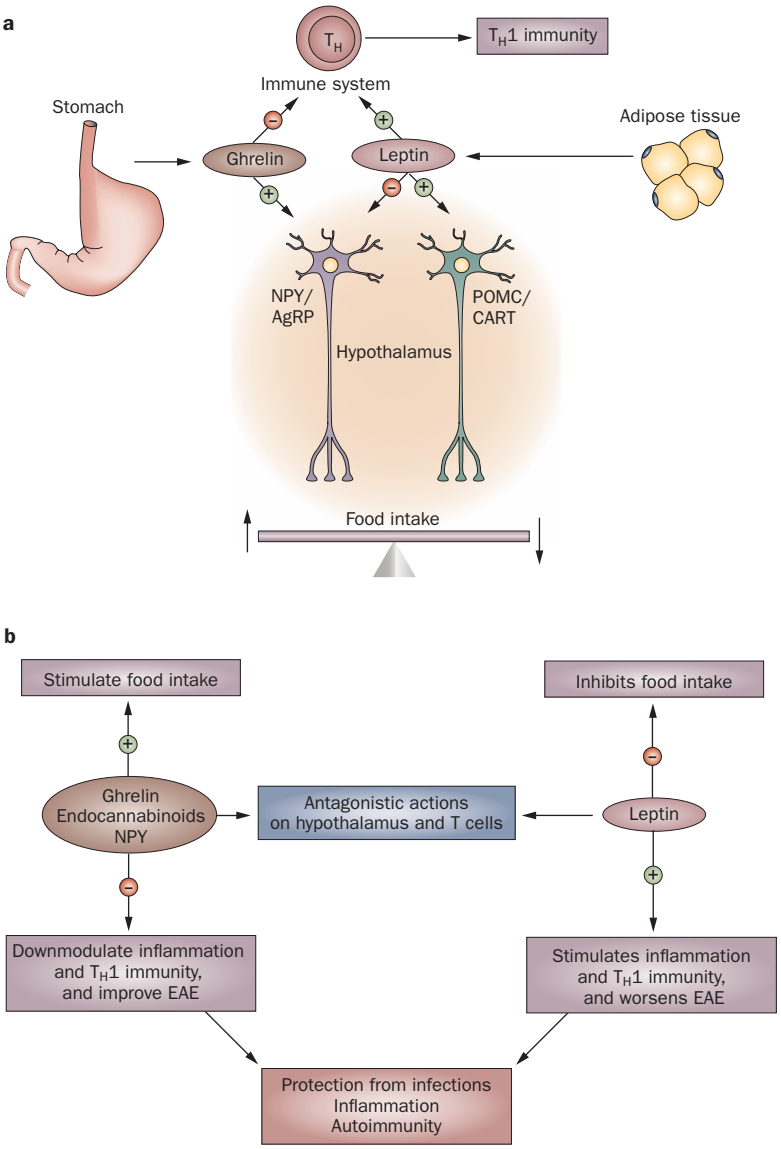

Figure 2 | Immune function and metabolic status. **a** | Leptin from the adipose tissue inhibits food intake by activating POMC/CART neurons and inhibiting NPY/AgRP neurons. By contrast, ghrelin from the stomach stimulates food intake by stimulating NPY/AgRP neurons.[46] **b** | Schematic diagram showing that leptin from the adipose tissue inhibits food intake and stimulates proinflammatory T_H1 immunity and EAE. Conversely, ghrelin, NPY and endocannabinoids stimulate food intake, inhibit the effects of leptin on the hypothalamus, and antagonize the proinflammatory effects of leptin on immune cells and EAE. Abbreviations: AgRP, agouti-related protein; CART, cocaine- and amphetamine-regulated transcript protein; EAE, experimental autoimmune encephalomyelitis; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; T_H, T helper.

Drugs that affect metabolism

Several pharmacological compounds that affect glucose and cholesterol metabolism and adipocyte development also have immunomodulatory activities (Box 2). Such effects are reflected in autoreactive T cells and pro-inflammatory immune responses in obesity, atherosclerosis and MS.[35–39]

Box 2 | Drugs that affect metabolism

The following drugs, all of which reduce leptin levels, are used to treat metabolic syndrome and insulin resistance, and have also been found to inhibit experimental autoimmune encephalomyelitis:

- Metformin (AMP-activated protein kinase agonist)[35]
- Thioazolinediones (peroxisome-proliferator activated receptor γ agonists)[36]
- Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors)[37]

Current strategies to treat obesity-related metabolic disturbances (such as insulin resistance and glucose tolerance) involve drugs that not only act on metabolism but are now also known to downregulate immune responses. Examples include metformin, thioazolinediones and statins, which are, to varying degrees, capable of reducing proinflammatory cytokine levels and leptin secretion (Box 2).[35–37] For example, metformin—the most widely used drug for type 2 diabetes, and the actions of which are mediated through the activation of the AMP-activated protein kinase AMPK—has anti-inflammatory properties and inhibits T-cell-mediated immune responses and the production of T_H1 or T_H17 cytokines, while inducing generation of IL-10-secreting T_{REG} cells.[35] In EAE, metformin reduces the clinical score and inflammation.

Thioazolinediones are potent activators of the transcription factor peroxisome-proliferator activated receptor γ (PPARγ) and are key players in the development and differentiation of adipose tissue. PPARγ agonists downregulate the transcription of proinflammatory cytokines such as leptin, TNF and IL-6. This class of drugs is used in the treatment of insulin resistance in obese and type 2 diabetic patients for its capacity to exert insulin-sensitizing actions.[36,38] In EAE, these drugs are able to stop disease progression and neural inflammation.[36] Finally, statins—inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase—inhibit cholesterol synthesis, thereby lowering cholesterol levels, and also dampen autoreactive immune responses by promoting the release of T_H2 cytokines and reducing the levels of proinflammatory cytokines such as leptin and TNF.[37,39] Statins are widely used in the treatment of type 2 diabetes and insulin resistance, and have been found to be highly effective as therapeutic agents in EAE.[39]

In summary, drugs that have long been considered to affect metabolism and insulin actions are also immune modulators that reduce proinflammatory cytokine levels and increase the number and function of T_{REG} cells and the release of T_H2 and regulatory-type cytokines, all of which are important in the control of autoimmunity.

Opposing roles of leptin in the CNS

As discussed above, data reported in the literature suggest that leptin is involved in the pathogenesis of both experimental autoimmune encephalomyelitis (EAE)—an animal model of MS—and human MS.[16] However, a dichotomous role of leptin on the CNS has emerged, with evidence suggesting that this protein can have differential effects on myelination, neural cell survival and immune-mediated demyelination at various stages of the life cycle.[40–42]

To study the role of leptin in neural development, investigators examined the effects of leptin deficiency and postnatal treatment with leptin on brain weight, neuronal and glial markers, and locomotor activity. Mice deficient in leptin or its receptors (*ob/ob* and *db/db* mice, respectively) displayed reduced brain weight and an immature pattern of expression of synaptic and glial proteins, including elevated levels of growth-associated protein in the neocortex and hippocampus, and decreased levels of syntaxin-1, synaptosomal-associated protein-25 (SNAP-25) and synaptobrevin throughout the brain.[40–42] Expression of myelin basic protein, PLP and glial fibrillary acidic protein was also decreased in the neocortex, hippocampus and striatum of *ob/ob* and *db/db* mice. Treatment of *ob/ob* mice with recombinant leptin increased brain weight and overall protein content, improved the locomotor activity of the animals, and normalized the levels of growth-associated protein, syntaxin-1 and SNAP-25, but had no effect on the expression of synaptobrevin or glial proteins.[40–42] These findings suggest that leptin is required for normal neuronal and glial maturation in the nervous system of the mouse.

The discovery that leptin has a dichotomous role in the CNS is not particularly surprising, as other cytokines such as IL-6, IL-7 and IL-15 can exert proinflammatory and demyelinating activities (through activation of self-reactive T cells) as well as promoting neural and myelin development.[43]

Conclusions and future perspectives

Leptin is considered to be an attractive immunotherapeutic target for reducing inflammation and autoimmunity. Conditions in which levels of leptin decrease dramatically, such as during the stress response of acute starvation, have been suggested to be beneficial in autoimmunity and detrimental during infection.[44,45] Leptin alone is unlikely to be sufficient to determine the outcome of autoimmunity or MS, but the observations that leptin neutralization can markedly delay the onset and progression of EAE and inhibit myelin-specific autoreactive T cells are important. These findings provide proof-of-principle evidence that leptin modulation in CNS autoimmunity—probably in combination with standard therapies—could increase treatment efficacy, target multiple proinflammatory pathways, and reduce drug dosages to levels that minimize adverse effects.

Review criteria

A comprehensive literature review was performed in PubMed (1970–2010), using the keywords "leptin" and "metabolism" in combination with one other search term to review major areas including the following: "T cell", "T_{REG}", "autoimmunity", "inflammation", "obesity", "multiple sclerosis", "EAE", "metabolism" and "nutrition". Only original articles in the English language were included.

1. Ahima, R. S. & Flier, J. S. Leptin. *Annu. Rev. Physiol*. **62**, 413–437 (2000).
2. La Cava, A. & Matarese, G. The weight of leptin in immunity. *Nat. Rev. Immunol*. **4**, 371–379 (2004).
3. O'Neill, L. A role for leptin in autoimmunity? *Trends Immunol*. **22**, 352 (2001).
4. Matarese, G. & La Cava, A. The intricate interface between immune system and metabolism. *Trends Immunol*. **25**, 193–200 (2004).
5. Steinman, L. A molecular trio in relapse and remission in multiple sclerosis. *Nat. Rev. Immunol*. **9**, 440–447 (2009).
6. Matarese, G. *et al.* Balancing susceptibility to infection and autoimmunity: a role for leptin? *Trends Immunol*. **23**, 182–187 (2002).
7. De Rosa, V. *et al.* A key role of leptin in the control of regulatory T cell proliferation. *Immunity* **26**, 241–255 (2007).
8. Sánchez-Margalet, V. *et al.* Role of leptin as an immunomodulator of blood mononuclear cells: mechanisms of action. *Clin. Exp. Immunol*. **133**, 11–19 (2003).
9. Hekerman, P. *et al.* Pleiotropy of leptin receptor signalling is defined by distinct roles of the intracellular tyrosines. *FEBS J*. **272**, 109–119 (2005).
10. Chan, J. L. *et al.* Differential regulation of metabolic, neuroendocrine, and immune function by leptin in humans. *Proc. Natl Acad. Sci. USA* **103**, 8481–8486 (2006).
11. Sanna, V. *et al.* Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses. *J. Clin. Invest*. **111**, 241–250 (2003).
12. Howard, J. K. *et al.* Leptin protects mice from starvation-induced lymphoid atrophy and increases thymic cellularity in *ob/ob* mice. *J. Clin. Invest*. **104**, 1051–1059 (1999).
13. Matarese, G. *et al.* Requirement for leptin in the induction and progression of autoimmune encephalomyelitis. *J. Immunol*. **166**, 5909–5916 (2001).
14. Sarraf, P. *et al.* Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *J. Exp. Med*. **185**, 171–175 (1997).
15. Lock, C. *et al.* Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat. Med*. **8**, 500–508 (2002).
16. Matarese, G. *et al.* Leptin increase in multiple sclerosis associates with reduced number of $CD4^+CD25^+$ regulatory T cells. *Proc. Natl Acad. Sci. USA* **102**, 5150–5155 (2005).
17. De Rosa, V. *et al.* Leptin neutralization interferes with pathogenic T cell autoreactivity in autoimmune encephalomyelitis. *J. Clin. Invest*. **116**, 447–455 (2006).
18. Farooqi, I. S. *et al.* Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J. Clin. Invest*. **110**, 1093–1103 (2002).
19. Härle, P. & Straub, R. H. Leptin is a link between adipose tissue and inflammation. *Ann. NY Acad. Sci*. **1069**, 454–462 (2006).
20. Frisullo, G. The effect of disease activity on leptin, leptin receptor and suppressor of cytokine signalling-3 expression in relapsing–remitting multiple sclerosis. *J. Neuroimmunol*. **192**, 174–183 (2007).
21. Munger, K. L., Chitnis, T. & Ascherio, A. Body size and risk of MS in two cohorts of US women. *Neurology* **73**, 1543–1550 (2009).
22. Gomez, R., Lago, F., Gomez-Reino, J., Dieguez, C. & Gualillo, O. Adipokines in the skeleton: influence on cartilage function and joint degenerative diseases. *J. Mol. Endocrinol*. **43**, 11–18 (2009).
23. Lago, F., Dieguez, C., Gómez-Reino, J. & Gualillo, O. Adipokines as emerging mediators of immune response and inflammation. *Nat. Clin. Pract. Rheumatol*. **3**, 716–724 (2007).
24. Taleb, S. *et al.* Defective leptin/leptin receptor signaling improves regulatory T cell immune response and protects mice from atherosclerosis. *Arterioscler. Thromb. Vasc. Biol*. **27**, 2691–2698 (2007).
25. Dixit, V. D. *et al.* Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J. Clin. Invest*. **114**, 57–66 (2004).
26. Theil, M. M. *et al.* Suppression of experimental autoimmune encephalomyelitis by ghrelin. *J. Immunol*. **183**, 2859–2866 (2009).
27. Bedoui, S. *et al.* Neuropeptide Y (NPY) suppresses experimental autoimmune encephalomyelitis: NPY_1 receptor-specific inhibition of autoreactive Th1 responses *in vivo*. *J. Immunol*. **171**, 3451–3458 (2003).
28. Malfitano, A. M. *et al.* Arvanil inhibits T lymphocyte activation and ameliorates autoimmune encephalomyelitis. *J. Neuroimmunol*. **171**, 110–119 (2006).
29. Piccio, L., Stark, J. L. & Cross, A. H. Chronic calorie restriction attenuates experimental autoimmune encephalomyelitis. *J. Leukoc. Biol*. **84**, 940–948 (2008).
30. Longo, V. D. & Fontana, L. Calorie restriction and cancer prevention: metabolic and molecular mechanisms. *Trends Pharmacol. Sci*. **31**, 89–98 (2010).
31. Esquifino, A. I., Cano, P., Jimenez, V., Cutrera, R. A. & Cardinali, D. P. Experimental allergic encephalomyelitis in male Lewis rats subjected to calorie restriction. *J. Physiol. Biochem.* **60**, 245–252 (2004).
32. Esquifino, A. I., Cano, P., Jimenez-Ortega, V., Fernandez-Mateos, M. P. & Cardinali, D. P. Immune response after experimental allergic encephalomyelitis in rats subjected to calorie restriction. *J. Neuroinflammation* **4**, 6 (2007).
33. Hewson, D. C. Is there a role for gluten-free diets in multiple sclerosis? *Hum. Nutr. Appl. Nutr.* **38**, 417–420 (1984).
34. Swank, R. L. & Dugan, B. B. Effect of low saturated fat diet in early and late cases of multiple sclerosis. *Lancet* **336**, 37–39 (1990).
35. Nath, N. *et al.* Metformin attenuated the autoimmune disease of the central nervous system in animal models of multiple sclerosis. *J. Immunol*. **182**, 8005–8014 (2009).
36. Diab, A. *et al.* Peroxisome proliferator-activated receptor-γ agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 ameliorates experimental autoimmune encephalomyelitis. *J. Immunol*. **168**, 2508–2515 (2002).
37. Youssef, S. *et al.* The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature* **420**, 78–84 (2002).
38. Heneka, M. T., Landreth, G. E. & Hüll, M. Drug insight: effects mediated by peroxisome proliferator-activated receptor-γ in CNS disorders. *Nat. Clin. Pract. Neurol*. **3**, 496–504 (2007).
39. Zhang, X. & Markovic-Plese, S. Statins' immunomodulatory potential against Th17 cell-mediated autoimmune response. *Immunol. Res*. **41**, 165–174 (2008).
40. Udagawa, J. *et al.* The role of leptin in the development of the cerebral cortex in mouse embryos. *Endocrinology* **147**, 647–658 (2006).
41. Valerio, A. *et al.* Leptin increases axonal growth cone size in developing mouse cortical neurons by convergent signals inactivating glycogen synthase kinase-3β. *J. Biol. Chem*. **281**, 12950–12958 (2006).
42. Ahima, R. S., Bjorbaek, C., Osei, S. & Flier, J. S. Regulation of neuronal and glial proteins by leptin: implications for brain development. *Endocrinology* **140**, 2755–2762 (1999).
43. Levine, J. M., Reynolds, R. & Fawcett, J. W. The oligodendrocyte precursor cell in health and disease. *Trends Neurosci*. **24**, 39–47 (2001).
44. Steinman, L., Conlon, P., Maki, R. & Foster, A. The intricate interplay among body weight, stress, and the immune response to friend or foe. *J. Clin. Invest*. **111**, 183–185 (2003).
45. Kuchroo, V. K. & Nicholson, L. B. Fast and feel good? *Nature* **422**, 27–28 (2003).
46. Gao, Q. & Horvath, T. L. Neurobiology of feeding and energy expenditure. *Annu. Rev. Neurosci*. **30**, 367–398 (2007).

Acknowledgments

G. Matarese is supported by grants from the European Union Ideas Program, ERC-Starting Independent Grant 'LeptinMS' project number 202579 and Telethon-JDRF Grant project number GJT08004. A. La Cava is supported in part by NIH grant AR53239. The authors thank Salvatore De Simone and Francesco D'Agnello for art graphics and the models in the figure. This work is dedicated to the memory of Eugenia Papa and Serafino Zappacosta.