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<td>(54) Title:</td>
<td>DIAGNOSTIC AND THERAPEUTIC METHODS RELATED TO REGULATING ENERGY MOBILIZATION WITH OB PROTEIN AND OB ANTIBODIES</td>
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<tr>
<td>(57) Abstract</td>
<td>Compositions comprising OB-R agonists and methods of treatment for conditions such as systemic inflammatory response syndrome are provided. One suitable OB-R agonist ligand is recombinant human OB protein, also known as leptin. Also provided are methods and compositions for the treatment of obesity and OB resistance. Assay methods and kits relating to these conditions are also included.</td>
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DIAGNOSTIC AND THERAPEUTIC METHODS RELATED TO
REGULATING ENERGY MOBILIZATION WITH
OB PROTEIN AND OB ANTIBODIES

Reference to Related Application

This application claims the benefit of U.S. Provisional Application
S.N. 60/018,972, filed June 4, 1996, which is incorporated by reference, as are
all references cited herein.

Governmental Rights

This invention was made with governmental support from the
United States Government, National Institutes of Health, Grant DK20043; the
United States Government has certain rights in the invention.

Background of the Invention

The *obese* gene in human, rat and mouse encodes a protein
hormone having an open reading frame 167 amino acid residues in length, called
leptin, also known as OB protein or the *ob* gene product. Removal of the signal
sequence yields a mature secreted 16 kilodalton protein that is 146 amino acid
residues in length.

OB protein is produced primarily by adipocytes of white adipose
tissue (WAT). OB protein is secreted directly into the extracellular space and
travels through the blood stream. OB protein affects the cells of its target organs
by binding to the OB receptor protein, OB-R, that is found on the extracellular
surface of the plasma membrane of target cells. Binding of OB protein-to OB-R
activates the intracellular second messenger cascade of the JAK-STAT system,
which is characteristic of activation of cytokine type I receptors.

OB protein is produced in adipocytes in proportion to the mass of
stored fat, thereby providing a hormone signal for a lipostatic feedback circuit,
which is mediated by the OB receptor. While OB proteins of different species
show a close similarity in their sequences, the sequences of OB proteins are not
closely similar to other types of proteins. For example, the human *ob* gene
sequence and its mouse homologue (85% sequence identity) have been reported to have no sequence similarity to other proteins of known structure (DiFrancesco, V., et al., Protein Topology Recognition from Secondary Structure Sequences: Application of the Hidden Markov Models to Alpha Class Proteins, J. Mol. Biol. 267: 446-463 (1997) at page 457).

Although OB protein is composed of a single peptide chain, an intrachain disulfide bond between cysteine 96 and cysteine 146 is required both to stabilize the conformation of the molecule and to confer in vivo biological activity (Rock, F.L., et al., The Leptin Haemopoietic Cytokine Fold is Stabilized by an Intrachain Disulfide Bond, Horm. Metab. Res. 28: 649-652 (1996)). It is believed that the special geometry of the A and D major helices must be maintained in order to dock to a conserved receptor trough in the receptor molecule, a requirement that produces structural similarity between OB proteins and cytokines in the face of negligible sequence conservation (Id. at 651.).

One accepted and successful animal model of human obesity is the genetically obese mouse bearing the recessive obese mutation (ob/ob). The mouse model reproduces not only the human obesity condition, but also develops non-insulin dependent diabetes mellitus (NIDDM, also known as type II diabetes mellitus). Homologous obese genes have been described in mouse, rat and human.

The mouse is also a widely accepted and successful model of sepsis, septic shock and systemic inflammatory response syndrome (SIRS), a term which describes the clinical syndrome of sepsis without regard to its cause. Simple models, involving a large bolus dose of lipopolysaccharide (LPS) administered to mice and using mortality as the primary outcome variable, are well suited for preliminary pharmacological studies of new drugs or other therapeutic agents (Fink, M.P. & Heard, S.O., Laboratory Models of Sepsis and Septic Shock, J. Surg. Res. 49: 186-196, 1990, at 188-189).


Neural activity in specific regions of the central nervous system (CNS), such as the hypothalamus, controls processes related to food intake and energy expenditure. The cloning of the OB protein gene and the OB receptor gene and the localization of OB receptor expression in the hypothalamus has provided supporting evidence for this view as well as suggesting possible mechanisms for relating food intake to stored fat reserves. The OB protein is produced by adipocytes in proportion to the mass of stored fat and, hence, it acts as the signal to a lipostat control circuit. This lipostat signal is transduced at the target cells by the OB receptor, OB-R, in the CNS, resulting changes in neural activity that regulate both food intake and metabolic rate.

Metabolic derangement is an important characteristic of the host response to critical illness called the acute phase response that characterizes conditions such as sepsis and septic shock (Kushner, I. Ann. N.Y. Acad. Sci. 389: 39-48 (1982)). Hypothermia is a metabolic response that may be pertinent clinical prognostic factor in systemic inflammatory response syndrome in humans (Brivet, F., et al. Crit. Care Med. 22: 533-534 (1994)).

There is a need for at least one disease marker for systemic inflammatory response syndrome (SIRS) and related conditions. Hereinafter, the term SIRS is used to denote sepsis, septic shock, sepsis syndrome, and related conditions. Disease markers have numerous functions. In this case, a marker for SIRS would be useful for predicting the development of SIRS, identifying patients
with SIRS, predicting outcome, aiding timing and targeting of therapeutic interventions, and determining the pathogenesis of SIRS in patients (Parsons, P.E. & Moss, M. Early Detection and Markers of Sepsis, Clinics in Chest Medicine 17:199-212 (1996)).

Summary of the Invention

Embodiments of the present invention include compositions and methods for treating a patient having a condition in which regulating energy metabolism during a systemic inflammatory response is desired, comprising administering a composition having a physiologically effective amount of at least one OB-R agonist ligand. Suitable OB-R agonist ligands include recombinant OB protein, peptide conformational analogs of human OB protein comprising conservative substitutions of amino acid residues and OB-related peptides. A preferred OB-R agonist ligand is recombinant human OB protein.

In another therapeutic embodiment, the beneficial aspects of OB-R agonist ligand administration are facilitated by a coordinated increase in the number of OB receptors (OB-R) produced by the administration of agents that OB-R expression inducers. Suitable OB-R expression inducers include lipopolysaccharide (LPS) and cytokines. Preferred cytokines are interleukin-1α (IL-1α), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6). Particularly preferred cytokines are IL-6 and IL-1β.

In a further therapeutic embodiment, antibodies to OB protein are used as agents capable of blocking the effects of OB receptor activation, especially fat mobilization and increased energy utilization. Suitable antibodies to OB proteins may be polyclonal or monoclonal. Suitable antibodies comprise immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antibody combining site or paratope. Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contain the paratope, including those portions known in the art as Fab, Fab' and F(ab')₂. This embodiment is suitable for
treating metabolic derangement due to conditions such as anorexia, amenorrhea, cachexia and the like.

Antibodies to OB protein are also useful as an assay kit and method for detecting the level of OB protein in a patient. The level of OB protein in a patient is a disease marker that is useful for predicting the development of a condition, identifying patients with the condition, predicting outcome of the condition, aiding timing and targeting of therapeutic interventions, and determining the pathogenesis of the condition in patients. Conditions in which the level of OB protein is a useful marker are SIRS and related conditions such as sepsis and septic shock, as well as anorexia, amenorrhea, cachexia and the like.

Brief Summary of the Drawings

In the drawings:

Figure 1 is a representation of an autoradiogram showing the results of a ribonuclease (RNase) protection assay showing expression of total OB receptor (OB-R_{o}) in lung, kidney and liver at 0, 4, 8 or 24 hours, or 2 (D_{2}), 3 (D_{3}) or 5 (D_{5}) days after intravenous injection of 5 μg per gram of body weight as well as a dose-response study showing the relative effects on the liver of injection of 0.05, 0.5 or 5 μg of LPS per gram of body weight;

Figure 2 is a representation of an autoradiogram showing of the results of a RNase protection assay showing expression of OB-Rb in normal liver (lane 1), and the LPS-treated liver at 24 hours (lane 2), and in the hypothalamus of normal control (lane 3) and ob/ob mice (lane 4), compared to OB-R_{m} which represents the mixture of OB-R forms partially protected by the designated nucleotide probes;

Figure 3 is a representation of an autoradiogram showing the results of a RNase protection assay showing expression of OB-Rc in normal liver (lane 1), and the LPS-treated liver at 24 hours (lane 2), and in the hypothalamus of normal control (lane 3) and ob/ob mice (lane 4), compared to OB-R_{m} which
represents the mixture of OB-R forms partially protected by the designated nucleotide probes;

Figure 4 is a representation of an autoradiogram showing the results of a RNase protection assay showing expression of OB-Re in normal liver (lane 1), and the LPS-treated liver at 24 hours (lane 2), and in the hypothalamus of normal control (lane 3) and ob/ob mice (lane 4), compared to OB-R\(_{\text{im}}\) which represents the mixture of OB-R forms partially protected by the designated nucleotide probes;

Figure 5 is a representation of an autoradiogram showing the results of a RNase protection assay showing OB-R expression in mouse liver 24 hours after IL-6 (2.5 \(\mu\)g per mouse), TNF-\(\alpha\) (10 \(\mu\)g per mouse) and IL-1\(\beta\) (5 \(\mu\)g per mouse) injection;

Figure 6 is a representation of an autoradiogram showing the results of a RNase protection assay showing OB-R mRNA levels in brain cortex, hypothalamus, and brain stem at various times after LPS injection (5 \(\mu\)g per gram of body weight);

Figure 7 is a representation of an autoradiogram showing the results of a RNase protection assay showing OB mRNA expression in the adrenal gland (Adr) and white adipose tissue (WAT) at various times after LPS injection (5 \(\mu\)g per gram of body weight);

Figure 8 is a graphical representation of the construction scheme for the vector pETM1 from a commercially available vector;

Figure 9 is a graphical representation of the weight gain induced by anti-OB antiserum injection in C57BL/6 mice which were given daily injections of anti-OB antiserum (antiOB) or preimmune rabbit sera (control), and whose body weight was measured 12 hours later, where data are expressed as mean \(\pm\) standard error of the mean (S.E.M), \(N=8\);

Figure 10 is a graphical representation of the time course of the survival of mice that had received a LPS injection (6 \(\mu\)g per gram of body weight) after pretreatment with either anti-OB protein antiserum (anti-OB,
N=16), preimmune rabbit serum (control, N=16), or one of three other unrelated rabbit antiserum (anti-X, N=9; 3 treated with each antiserum);

Figure 11 is a graphical representation of the time course of the survival of mice that were treated with OB protein (mOB, N=16) or vehicle (control, N-16) after a LPS injection (10 μg per gram of body weight);

Figure 12 is a graphical representation of the time course of the change in body temperature of mice that had received a LPS injection (6 μg per gram of body weight) after pretreatment with either anti-OB protein antiserum (anti-OB, N=16, except at * where N<16 due to mortality) or preimmune rabbit serum (control, N=16), data expressed as mean ± S.E.M.;

Figure 13 is a graphical representation of the time course of the change in body temperature of mice that were treated with OB protein (mOB, N=16) or vehicle (control, N-16, except at * where N<16 due to mortality) after a LPS injection (10 μg per gram of body weight), data expressed as mean ± S.E.M.;

Figure 14 is a graphical representation of the time course of the change in body weight (percent of initial body weight) of mice that had received a LPS injection (6 μg per gram of body weight) after pretreatment with either anti-OB protein antiserum (anti-OB, N=16) or preimmune rabbit serum (control, N=16, except at * where N<16 due to mortality), data expressed as mean ± S.E.M.;

Figure 15 is a graphical representation of the time course of the change in body weight (percent of initial body weight) of mice that were treated with OB protein (mOB, N=16) or vehicle (control, N-16, except at * where N<16 due to mortality) after a LPS injection (10 μg per gram of body weight), data expressed as mean ± S.E.M.; and

Figure 16 is a representation of an autoradiogram showing the results of a RNase protection assay showing the expression of iNOS, IL-1α, IL-1β, and TNF-α mRNAs in mouse.
Detailed Description of the Preferred Embodiments

It has been found that substances that initiate or mediate SIRS, for example, LPS and several cytokines, induce the increased expression of OB-R in liver and other peripheral tissues. Thus, occupancy and activation of OB-R by an agonist ligand such as recombinant OB protein, OB-related peptides or peptide conformational analog of human OB protein comprising conservative substitutions of amino acid residues serves as a protective homeostatic mechanism in systemic inflammatory response syndrome conditions such as endotoxic shock, sepsis and septic shock. A preferred OB-R agonist ligand is recombinant OB protein.

Suitable therapeutic human doses of recombinant OB protein are from about 1 micrograms per kilogram body weight to about 50 microgram per kilogram body weight. One preferable therapeutic human dose is about 10 micrograms per kilogram body weight.

While the regulation of energy homeostasis is essentially a function of the CNS, food intake and the majority of the energy expenditure take place in peripheral organs such as the liver. It has been found that the OB protein and the OB receptor have a functional involvement in peripheral energy homeostasis. In general, critical illness and trauma can dramatically alter metabolism, with the expression of the OB receptor changing in response to pathological stress. The expression of OB-R in liver and other peripheral organs, but not in the central nervous system, has now been shown to be induced by endotoxic shock produced intravenous injection of cytokines, such as, IL-1β, TNF-α and IL-6, as well as cytokine inducing agents such as LPS, into mice, an accepted animal model of SIRS and related conditions. OB protein, antibodies to OB protein, and OB-R expression inducers are useful for the diagnosis and treatment of conditions such as sepsis, systemic inflammatory response syndrome, cachexia and anorexia.

The administration of recombinant mouse OB protein to mice following OB-R induction with a normally lethal dose of LPS conferred complete resistance to LPS, resulting in survival. The OB-treated mice maintained a higher body temperature and displayed dramatic weight loss in contrast to control
counterparts. In vivo administration of OB antisera, on the other hand, elicited the opposite effects by blocking OB-mediated processes, thereby stimulating post-prandial food intake leading to rapid weight gain. Co-administration of LPS with a second in vivo treatment with OB antisera, however, resulted in 100% mortality as compared to animals treated with control antisera.

OB protein, in mediating host responses to LPS-induced endotoxemia, exerts its protective effect primarily by initiating energy mobilization and heat production in critical conditions, the effect of which is proportional to the level of OB protein in the blood. By altering the levels of OB protein, the amount of energy mobilized to resist challenges induced by inflammatory agents is correspondingly altered, thereby effecting the ultimate inflammatory response.

Therefore, in view of the newly discovered physiological properties of OB protein and OB antibodies in regulating energy mobilization and consumption, the present invention describes both diagnostic and therapeutic methods relating to the use of recombinant human OB protein and antibodies thereto in modulating the effects of OB-R activation in vivo.

Diagnostic Applications

OB antibodies are useful for detecting the amount of OB present in sample taken from a patient. One preferred diagnostic embodiment is the use of OB antibodies for detecting the amount of OB present in a blood sample taken from a patient exhibiting a SIRS condition such as sepsis, septic shock, and the like. Such measurements of OB levels in vitro in blood sample by antibody detection is also indicated in various wasting conditions or syndromes associated with several disease states or syndromes, including anorexia, amenorrhea, cachexia, chronic inflammatory conditions, AIDS and AIDS-related conditions, as well as sepsis, septic shock, SIRS and the like. Alternatively, the determination of OB levels is useful in systemic inflammatory response syndromes (SIRS) that are characterized by an acute increase in inflammatory mediators, such as IL-1β, IL-6, TNF, LPS and the like. Such conditions are noted in preoperative patients
subject to fasting, in patients with acute injuries such as burns or trauma, in patients with SIRS, or with ongoing bacterial infections or those receiving TNF-α for treatment of tumors and in persons suffering from hypothermia.

While there is little sequence similarity between OB proteins and other molecules, the three-dimensional conformation of the OB protein molecule is analogous to that of several long-chain helical cytokines: four major alpha helix regions, A-D, connected by short loops and minor helical regions (Zhang, F., et al., Crystal Structure of the obese protein leptin-E100, *Nature* 387: 206-209 (1997)).

As used herein, a conformational analog of OB protein is a molecule having substantially the same conformational characteristics of its three-dimensional structure that are required for activation of the OB receptor. Examples of such conformational characteristics include the conformation of the A major helix, the conformation of the D major helix, and the disulfide bond that maintains the geometrical relationship between the A and D major helices. Thus, amino acid substitutions that conserve the conformational characteristics of the molecule, for example, in the loop regions connecting the major helices, would produce conformational analogs to OB protein.

Peptides derived from the region of the OB protein from amino acid residues 106 to 140, as short as about 15 amino acids long, have been shown to be effective in mimicking the action of full-length recombinant OB protein (Grasso, P., et al., *In vivo* Effects of Leptin-Related Synthetic Peptides on Body Weight and Food Intake in Female ob/ob Mice: Localization of Leptin Activity to Domains Between Amino Acid Residues 106 -140, *Endocrinology* 138: 1413-1418 (1997)). As used herein, “OB-related peptides” refers to natural or synthetic peptides derived from the region of the OB protein from about amino acid residue 106 to about amino acid residue 140 and includes conservative amino acid residue substitutions.

The term “antibody” in its various grammatical forms is used herein to refer to immunoglobulin molecules and immunologically active portions
of immunoglobulin molecules, i.e., molecules that contain an antibody combining site or paratope. Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contain the paratope, including those portions known in the art as Fab, Fab’ and F(ab’)2. An antibody composition of the present invention is characterized as containing antibody molecules that immunoreact with OB protein or portions thereof.

An antibody composition of the present invention is typically produced by immunizing a mammal with a inoculum of OB protein or some fragment of OB protein, alone or in combination with a suitable adjuvant such as Freund’s adjuvant, and thereby induce in the mammal antibody molecules having the appropriate immunospecificity. The antibody molecules are then collected from the mammal and isolated to the extent desired by well known techniques such as, for example, immunoaffinity chromatography. The antibody composition so produced can be used, inter alia, in the diagnostic methods and systems of the present invention or in the preparation of therapeutic compositions of the present invention.

Monoclonal antibody compositions can also be used with the present invention. A monoclonal antibody composition contains, within detectable limits, only one species of antibody combining site capable of effectively binding to OB protein. Thus, a monoclonal antibody composition of the present invention typically displays a single binding affinity for OB protein even though it may contain antibodies capable of binding proteins other than OB protein. Preferred monoclonal antibodies are those that bind to portions of the OB protein that are required for activation of the OB receptor, such as the A helix, the D helix, or regions of OB protein that maintain the relative positions of the A and D helices that are required for the activation of the OB receptor. Monoclonal antibodies against human OB protein have been described and their preparation was discussed in (Tsuruo, Y. et al., Horm. Metab. Res. 28: 753-755 (1996). Monoclonal antibodies are also supplied commercially from vendors on a custom

Determination of OB levels with OB antibodies is performed by assay methods, including ELISA, radioimmunoassay (RIA), Western blot analysis, and the like, that are familiar to one of ordinary skill in the art. The determined OB protein levels are then compared to normal levels for the state of the patient, e.g., fasting, time of day, body mass index (BMI), aerobic conditioning, gender, etc. For example, the normal range found for lean males at 8 a.m. was 12.0 ± 4.4 ng/ml (Sinha, M.K., et al., Nocturnal Rise of Leptin in Lean, Obese, and Non-Insulin-dependent Diabetes Mellitus Subjects, J. Clin. Invest. 97: 1344-1347 (1996)). See, also Horn, R. et al., Radioimmunoassay for the detection of leptin in human serum, Exp. Clin. Endocrinol. Diabetes 104: 454-458 (1996); McGregor, G.P., et al., Radioimmunological Measurement of Leptin in Plasma of Obese and Diabetic Human Subjects, Endocrinology 137: 1501-1504 (1996). It has recently been found that OB protein is present in the circulation in both bound and free form, and that the ratio of the two forms is different in lean and obese subjects (Sinha, M.K., et al., Evidence of Free and Bound Leptin in Human Circulation. Studies in Lean and Obese Subjects and During Short-Term Fasting, J. Clin. Invest. 98: 1277-1282 (1996)). The relation of free and bound forms to OB protein biological activity can be considered in the context of OB protein assays.

In an alternative embodiment, immunohistochemical assay of OB-R receptor numbers are performed on tissue biopsy materials using standard protocols. A preferred tissue biopsy is liver biopsy.
Therapeutic Applications

Embodiments of the present invention, including methods of administering to a patient compositions comprising OB-R agonist ligands are useful in treating conditions in which it is desirable to regulate or modify energy metabolism during a systemic inflammatory response. Suitable OB-R agonist ligands include recombinant OB protein, peptide conformational analogs of human OB protein comprising conservative substitutions of amino acid residues and OB-related peptides. A preferred OB-R agonist ligand is recombinant human OB protein. A suitable dosage range for recombinant human OB protein is from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight. OB-related peptides are used in a dosage range from about 0.1 microgram per kilogram body weight to about 5 micrograms per kilogram body weight, adjusting the dosage to account for art-recognized differences in potency and solubility (Grasso, P. et al., (1997)).

In a related embodiment, compositions comprising at least one OB-R expression inducer are useful for treating obesity and conditions in which there is an insufficient number of OB receptors, in which low copy number of OB receptors is a limiting factor or in which there is "OB resistance," i.e., a reduced effect of associated with a particular plasma concentration of OB protein.

In general, the OB-R expression inducer is administered in an amount from about 0.003 to about 20 micrograms per kilogram body weight. Suitable OB-R expression inducers include therapeutic cytokines used in cancer therapy, such as IL-1α, IL-1β, IL-6 and TNF-α. Suitable dosages and modes of administration are known in the art. For example, a suitable dosage ranges for IL-1α is about 0.1 to about 6 micrograms/m²/day. A suitable dosage range for IL-1β is about 3 to about 200 nanograms/kg/day. A suitable dosage range for IL-6 is about 0.5 to about 20 micrograms/kg/day, with a preferred dosage range for IL-6 being about 1 to about 5 micrograms/kg/day. The therapeutic cytokines may be administered singly or in combination. The mode of administration may be intravenous infusion over an extended time period or a single intravenous or
subcutaneous injection. The daily dose may be administered as a single dose or divided into multiple doses given at intervals during the day.

In a further embodiment, to counteract the possible toxic side effects of OB-R expression inducers such as therapeutic cytokines, such substances are administered in a composition in combination with OB-R agonist ligands. Administration of such compositions is useful for conditions in which cytokines are normally administered for a therapeutic purpose such as tumor treatment, in order to provide effective protection by the OB-R agonist ligands from undesirable metabolic side effects. The up-regulation of the OB-R allows for the complete therapeutic effect mediated by OB-R agonist ligands such as OB protein. A suitable dosage range for recombinant human OB protein is from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

In an alternative embodiment, the present invention describes the administration of OB antisera, polyclonal or monoclonal, for treating conditions marked by increased OB and/or increased OB-R activity. Such conditions are various wasting conditions or syndromes associated with several disease states or syndromes, including anorexia, amenorrhea, cachexia, chronic inflammatory conditions, AIDS and AIDS-related conditions. A suitable dosage range for anti-OB protein antibodies is about 0.02 to about 15 milligrams / kg / day.

It has been found that OB protein is an important host defense factor against endotoxin stress. The protective effect of OB protein against endotoxin was not caused by suppressing the expression of major inflammatory mediators, since the mRNA levels of IL-1α, IL-1β, TNF-α, and iNOS in lung and spleen were similar in all LPS-treated mice regardless of the experimental manipulations (Figure 16). A comparison of the four groups of mice revealed striking correlations among the OB protein available, the survival from endotoxin shock (Figures 10 and 11), the maintenance of body temperature (Figures 12 and 13), and the loss of body weight (Figures 14 and 15).
Anti-OB Ab-treated mice showed the least loss of body weight and had the most profound hypothermia even with a relatively low dose of LPS. Conversely, OB-treated mice receiving a high dose of LPS maintained a higher body temperature than those control mice that received less LPS. The OB-treated mice also had a greater weight loss than any reported in the literature (16% in the first 24 hours, compared with an average of 10% reported by other groups). The mice in these tests were age, sex and weight matched, fed the same diet, and, therefore, should have had very similar energy store. The different responses to endotoxemia described were likely due to differences in metabolic energy mobilization and dissipation, which, in turn, were attributed to the experimental manipulation of circulating OB protein levels.

When the level of circulating OB protein is varied, the energy mobilized to resist endotoxin challenge is correspondingly altered, and the outcome of the host response to endotoxin stress is affected. \( ob/ob \) mice, lacking OB protein due to a mutation in the \( ob \) gene, were very sensitive to LPS insult: a dose as low as 2 \( \mu \)g per gram of body weight caused a rapid fall of body temperature and death.

The results also suggest the existence of two pathways of thermogenesis and thermostasis. Anti-OB Ab treatment per se did not cause hypothermia in normal mice, suggesting that the thermostasis under non-pathological conditions was largely OB protein-independent. However, when given LPS, the anti-OB Ab-treated mice developed profound hypothermia, indicating that the thermogenesis in response to endotoxemia had become OB protein dependent. A corollary of this model is that genetic defects affecting the OB/OB-R pathway will have a severe hypothermic response to endotoxin. Indeed, \( db/db \) mice which carry a mutation in the OB-R gene, responded to a low dose of LPS injection in a manner very similar to that seen in \( ob/ob \) mice (data not shown), despite their increased level of OB expression.
Example 1: Induction of OB-R Expression by Administration of LPS

The injection of LPS and cytokines, substances that are associated with sepsis, septic shock or SIRS caused increases in the expression of the OB receptor in peripheral organs such as liver, but not in brain.

Intravenous injection of lipopolysaccharide (LPS), IL-1β, TNF-α and IL-6 to mice induced OB receptor expression in the liver and other peripheral organs, but not in the central nervous system (CNS). To investigate the functional significance of the increased OB-R expression, an anti-OB antiserum was used to neutralize endogenous OB protein in mice prior to an LPS injection.

The neutralization of OB protein led to profound hypothermia, insignificant loss of body weight, and death in mice in response to an otherwise nonlethal dose of LPS. Conversely, mice administered recombinant mouse OB protein became more resistant to LPS and survived an otherwise lethal dose. The OB protein-treated mice maintained a relatively high body temperature and displayed a dramatic weight loss. These results suggest that OB protein may promote energy mobilization to compensate for the increased energy consumption in endotoxemia, and that the OB/OB-R pathway may play an important role in critical host responses to inflammatory stress.

Methods:

In general, standard techniques or published modifications were used; see, generally, Sambrook, J., et al., Molecular Cloning A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press (1989). C57BL/6 mice, 5-8 weeks of age and 17-20 g of weight, were injected intravenously with either LPS (5 μg per gram of body weight, List Biological Laboratory, Campbell, CA), IL-1β (R&D Systems, Minneapolis, MN), IL-6 (2.5 μg, Pharmingen, San Diego, CA), or TNF-α (a gift from Genentech, San Francisco, CA). Animals were sacrificed at 0, 4, 8 or 24 hours, or 2, 3 or 5 days after the injection.

Tissue from various organs, including brain, liver and kidney, was dissected and snap-frozen in liquid nitrogen. Total RNA was prepared from the frozen tissues by a single-step method (Chomczynski, P. & Sacchi, N., Single-

The RNase protection assays were carried out as previously described (Feng, L., et al., Alternative Splicing of the NC1 Domain of the Human α3(IV) Collagen Gene *J. Biol. Chem.* 269: 2342-2348 (1994); Xia, Y., et al., LPS-Induced MCP-1, IL-1β, and TNF-α mRNA Expression in Isolated Erythrocyte-Perfused Rat Kidney, *Am. J. Physiol.* 264: F774-F780 (1993)). A ten microgram aliquot of total RNA pooled from three similarly treated mice was used for each sample in the RNase protection assay. The stored pooled samples were dissolved in 10 μl of 80% formamide, 0.4 M NaCl, 1 mM EDTA, and 40 mM piperazine-N,N'-bis(2-ethanesulfonic acid), heated to 85 degrees Celsius for 5 minutes. Each ten microgram sample was then hybridized with about 1 x 10⁶ cpm (counts per minute) of the appropriate [³²P]UTP-labeled antisense riboprobe at 55 degrees Celsius for at least 10 hours. The unhybridized RNA was then digested with 50 unit/ml RNase T1 (GIBCO/BRL, Gaithersburg, MD) and 24 μg/ml RNase A at 30 degrees Celsius for one hour. The RNase was then digested with 625 μg/ml proteinase K (Boehringer Mannheim, Indianapolis, IN) for 30 minutes at 37 degrees Celsius. After phenol-chloroform extraction and sodium-acetate-ethanol precipitation, the protected hybridized RNA was denatured and electrophoresed on a 10% polyacrylamide gel. The gels were transferred to 3M Whatman filter paper, dried and exposed to Kodak X-Omat film. The resulting autoradiograms were developed in a Kodak X-Omat processor were used only for qualitative screening.

Radioactivity due to hybridization of target sequences with [³²P]-labeled riboprobes was quantified by scanning the gels on an AMBIS radioanalytic scanning system (AMBIS Systems, San Diego, CA).

An OB-Rb cDNA probe (from base 2548 to base 2835 of OB-Rb, Gen-Bank™ Accession No. U46135) was subcloned from a full-length mouse OB-Rb cDNA. The full-length mouse cDNA for the OB-R long form (OB-Rb)
was cloned from a mouse hypothalamus cDNA library (Stratagene, La Jolla, CA), and the sequence was verified against that of U46135.

The full-length mouse cDNA for the OB-R short form (OB-Ra) was cloned from a mouse lung cDNA Library (Stratagene, La Jolla, CA). A 224 bp probe that included base 1250 to base 1474 (as indicated on the OB-Rb sequence) of OB-Ra was used for the RNase protection assay. This fragment, which comprises a sequence that is shared by all variants of OB-R, was used as a probe for the total level of OB-R (OB_{R0}).

The expression of other forms of OB-R mRNA was analyzed using selective probes for the respective different forms of OB-R. The designated probes provided full protection to their corresponding OB-R forms and partial protection for other OB-R forms. A probe derived from L32 (33-126, Gen-Bank™ Accession No. X06483), a housekeeping gene encoding ribosomal protein, was used as a control.


One suitable protocol for RT-PCR is a modification of that previously described (Feng, L., et al., J. Biol. Chem. 269: 2342-2348, 1994). The primers used in RT-PCR are listed in Table 1, below. Primer oligonucleotides were synthesized using an ABI model 380B synthesizer (Applied BioSystems, Foster City, Ca).
Table 1: PCR Primers

<table>
<thead>
<tr>
<th>Sequence</th>
<th>(SEQ ID NO.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OB-Rc sense 5'-GCTATCGACAAGCAGCAGAAT-3'</td>
<td>8</td>
</tr>
<tr>
<td>OB-Rc antisense 5'-TGAACACAAACAACATAAGCCC-3'</td>
<td>9</td>
</tr>
<tr>
<td>OB-Re sense 5'-TGTATATCTGGTTATTATGAGAATGG-3'</td>
<td>10</td>
</tr>
<tr>
<td>OB-Re antisense 5'-CATTAATGATTATTATCGAATTGC-3'</td>
<td>11</td>
</tr>
</tbody>
</table>

First strand cDNA synthesis was performed using total liver RNA from LPS-treated C57BL/6 mice and murine leukemia virus reverse transcriptase with a random hexanucleotide primer. The 100 μl reaction mixture contained standard enzyme buffer, 5 μg of total RNA, 20 units of RNasin (RNase inhibitor), 500 pmol of hexanucleotide primer, 10 mM dithiothreitol, 1 mM of each dNTP, with 200 units of reverse transcriptase. Each reaction mixture was heated to 95 degrees Celsius for 10 minutes. PCR was then performed with separate aliquots of the reaction mixture with the appropriate primers for 35 cycles, using 60 degrees Celsius for annealing.

The cDNA segments used to generate riboprobes were excised by the appropriate restriction endonucleases and subcloned into the multiple cloning site of a standard transcription vector. Suitable transcription vectors include a vector chosen from the pGEM series (Promega, Madison, WI). Labelled single stranded riboprobes were synthesized using standard in vitro transcription protocols, either those provided by the manufacturer or other standard protocols (e.g. Ausubel, F.M., et al., pages 4-18 - 4-21) with the appropriate (e.g., SP6 or T7) bacteriophage RNA polymerase. The riboprobes contained regions corresponding to the vector poly linker in addition to the region corresponding to the target sequence, and thus were longer than the protected bands. The mouse ribosomal L32 gene, a constitutively expressed "housekeeping" gene, was used throughout the study as a control.
Results:

When LPS was administered to C57BL/6 mice, a strong induction of total OB-R expression (OB-R_{0}) was detected in a number of peripheral organs (Figure 1), but not in several areas of the central nervous system, such as the hypothalamus, that are known to express OB-R (compare Figure 1 and Figure 6). The increased expression of OB-R was most prominent in the liver, the major site of metabolic regulation. The increase of OB-R mRNA expression in the liver was LPS dose-dependent, and peaked between 24 and 48 hours post-LPS injection (Figure 1).

Unexpectedly, RNase protection assays using probes specific for alternatively spliced forms of OB-R mRNA revealed that the long form, OB-Rb, was also induced in the liver to a level comparable to that found in the ob/ob mouse hypothalamus and greater than that of the lean control mouse hypothalamus (Figure 2). However, the majority of hepatic OB-R were the OB-Ra, OB-Rc (Figure 3) and OB-Re (Figure 4) forms. OB-Rd expression in the liver was undetectable (data not shown).

In addition to LPS, OB-R expression was induced by the cytokines IL-6, IL-1α and TNF-α (Figure 5). Contrary to a recent report (12), we found no detectable increase in OB expression in white adipose tissue in LPS-treated mice, but detected a distinct induction of OB mRNA expression in the adrenal gland (Figure 4). No OB mRNA expression was found in the brain, heart, lung, liver, kidney, spleen, muscle, stomach, duodenum, jejunum, ileum, or colon of LPS-treated mice (data not shown).

Example 2: Production of Recombinant OB Protein

Recombinant OB protein was expressed in E. coli using a prokaryotic expression vector and extracted from inclusion bodies. Other vectors and host cells systems, including eukaryotic cells, are known in the art and also suitable for the expression of OB protein. See, generally, Ausubel, F.M., et al. Short Protocols in Molecular Biology, 2nd Ed., pages 16-1 to 16-89.
The coding region of mouse OB cDNA (65-619, Gen-Bank™ Accession No.U18812) was cloned by RT-PCR of total RNA from C57BL/6 white adipose tissue. The coding region was subcloned in expression vector, pETM1 (Feng, L., et al., *J. Biol. Chem.*, 269: 2342-2348, 1994), to express a His-tagged recombinant mouse OB protein. The construction of pETM1 from the commercially available vector pET-11a (Novagen, Madison, WI) is illustrated on Figure 8.

After the expression of OB protein is induced, the bacteria were harvested and the inclusion bodies were extracted with a buffer containing 6M urea. The extract was loaded on a Ni-NTA affinity column (Qiagen, Chatsworth, CA) and the purification procedure was carried out as previously described (Feng, et al.(1994)). The protein was refolded on the column by adding refolding buffer containing 5 mM CaCl₂/20 mM Tris/0.2 NaCl with an urea gradient of 4 M - 0.5 M at a rate of about 0.5 ml/minute. After refolding, the protein was eluted with 80 mM imidazole/5 mM CaCl₂/20 mM Tris/0.2 NaCl/0.5 mM urea and then dialyzed against phosphate-buffered saline (PBS). Polyclonal antibodies was raised by immunizing a rabbit with the recombinant mouse OB and Freund’s adjuvant using standard procedures. Antiserum was used in the following examples.

**Example 3: Effects of Anti-OB Antibodies on Metabolism**

Intravenous administration of antibodies directed against OB protein effectively opposes the effects of endogenous OB protein. Recombinant mouse OB protein was produced in an *E. coli* expression system as described in Example 2, and was used to generate rabbit polyclonal anti-OB antibody. The antibody, when injected intravenously into mice, stimulated food intake, leading to rapid weight gain, and thus was effective in blocking OB protein function. The results are shown in Figure 9.

Female C57BL/6 mice, 6-8 weeks of age and 15-17 g of weight, were group housed four per cage and adapted to a 12:12 hour light: dark cycle (light from 6:00 to 18:00). Mice were given a daily intravenous injection of 0.2
ml anti-OB antiserum or preimmune rabbit sera ("vehicle") at 10 p.m., after their initial dark phase food intake. Their body weight was measured at 10 a.m. the next day.

The weight gain induced by anti-OB antiserum is illustrated in Figure 9. Data are expressed as mean \pm S.E.M. (N=8). While the weight of the control group remained essentially constant over the week, the anti-OB treated group showed a weight increase at the first weighing, which continued for the entire study period.

Example 4: Effects of Anti-OB Antibodies and Recombinant OB-Protein on Response to Endotoxic Shock

The fact that the OB-R variants induced in the liver were predominantly short forms raised the question of functional relevance of the hepatic OB-R expression. OB-Rb is the main form expressed in the hypothalamus, while the choroid plexus expresses only OB-Ra. That the mutation in \textit{db/db} mice affects OB-Rb, but not OB-Ra, suggests that OB-Rb is crucial for regulating food intake and OB-Ra may act as an OB protein transporter. Accordingly, the prominent expression of OB-R in the liver could initiate intracellular signal transduction or, alternatively, mediate the clearance of OB protein. We found that administration of neutralizing anti-OB antibody (Ab) or OB protein to LPS-treated mice distinguished between the two alternatives.

Male C57BL/6 mice, 5-8 weeks of age and 17-21 g of body weight, were used for this study. For antisera treatment, mice were given an i.v. injection of 200 \( \mu l \) rabbit antisera. LPS at a dose of 6 \( \mu g \) per gram of body weight was then co-injected with a second dose of anti-OB antibody to the pretreated mice. LPS was dissolved in antisera at a concentration of 0.6 mg/ml and was injected intravenously 4 hours after the initial antisera treatment. Food was retrieved from mouse cages during the 4-hour pretreatment period to prevent any food intake differences resulting from anti-OB antiserum-induced hyperphagia, and was added back after LPS injection.
While this dose of LPS was not lethal to C57BL/6 mice treated with preimmune rabbit serum, all the mice in the anti-OB Ab-treated group died within 40 hours. Figure 10 illustrates the results from three groups of mice: those pretreated with anti-OB antiserum (anti-OB, N = 16), preimmune rabbit sera control, N = 16), or three other unrelated rabbit antisera ("Anti-X", N = 3 for each antiserum). This LPS sensitizing effect was specific for anti-OB Ab, since mice treated similarly with three other unrelated antibodies ("Anti-X") all survived (Figure 10).

In comparison, mice treated with OB protein (5 µg per gram of body weight mOB, N = 16) were able to survive a higher dose of LPS (10 µg per gram of body weight) that was fatal to the control group of mice receiving the vehicle alone (control, N = 16). Mouse OB protein and LPS were prepared in saline at a concentration of 0.5 mg/ml and 1 mg/ml, respectively, and injected intravenously into the mice. A vehicle solution used for OB protein dialysis was injected into control mice. To eliminate any circadian effect, experiments at different days were all started at the same hours. Mice were examined at 4-hour intervals post LPS injection for the first 24 hours, and survival was monitored for 7 days following the LPS injection. The results are shown in Figure 11. The dose of LPS killed all the mice in the control group within 24 hours. However, in the experimental group, OB protein treatment conferred mice complete resistance to this dose of endotoxin. The OB-treated mice displayed noticeably less severe symptoms of endotoxemia, remaining alert and responsive to touch and other manipulation, and recovering quickly.

The effects of both OB protein and anti-OB antibodies on body temperature and body weight were monitored in the same groups of mice. Core body temperature measurements were made by insertion of a thermistor probe (Yellow Springs Instrument, Yellow Springs, Ohio) into the colon, 1.5 cm beyond the rectum. Body weight measurements were made on a portable digital balance (Ohaus, Florham Park, NJ).
The mice receiving anti-OB antibodies, which ultimately died, showed lower body temperature (Figure 12) and less weight loss (Figure 14) than the corresponding control group than survived. Conversely, the mice receiving OB-protein, and which survived the endotoxic shock, showed higher body temperature (Figure 13) and more weight loss (Figure 15) than the corresponding control group which succumbed.

While administration of both OB protein and anti-OB antibodies had significant effects on survival, body weight and body temperature, there was little effect on the expression of iNOS, IL-1α, IL-1β, and TNF-α mRNAs in lung and spleen (Figure 16). Mice were treated as described in above (Figures 10-15).

RNase protection assays were performed as described in Example 1. Each sample was 5 μg total RNA was used for each sample in the RNase protection assay. Riboprobes were produced as described in Example 1 based on the following cDNA fragments: IL-1α (from base 172 to base 366, Accession No. X01450, SEQ ID NO. 3), IL-1β (from base 500 to base 671, Accession No. M15131, SEQ ID NO. 4), TNF-α (from base 428 to base 557, Accession No. M11731, SEQ ID NO. 5), mouse iNOS (from base 2404 to base 2698, Accession No. M92649, SEQ ID NO. 6), and L32 (from base 33 to base 126, Accession No. X064383, SEQ ID NO. 7).

Relatively little change in the pattern of expression of these markers was observed (Figure 16), suggesting that these the protective actions of OB proteins are direct and not indirect and mediated by these cytokines.

The foregoing is intended to be illustrative of the present invention, but not limiting. Numerous variations and modifications of the present invention may be effected without departing from the true spirit and scope of the invention.
(1) GENERAL INFORMATION:

(i) APPLICANT: Feng, Lili
    Chen, Sizhong
    Xia, Yiyang

(ii) TITLE OF INVENTION: DIAGNOSTIC AND THERAPEUTIC METHODS
    RELATED TO REGULATING ENERGY MOBILIZATION WITH OB PROTEIN
    AND OB ANTIBODIES

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
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    (C) CITY: Chicago
    (D) STATE: IL
    (E) COUNTRY: US
    (F) ZIP: 60606

(v) COMPUTER READABLE FORM:
    (A) MEDIUM TYPE: Floppy disk
    (B) COMPUTER: IBM PC compatible
    (C) OPERATING SYSTEM: PC-DOS/MS-DOS
    (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
    (A) APPLICATION NUMBER:
    (B) FILING DATE: 04-JUN-1997
    (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
    (A) APPLICATION NUMBER: US 60/018,972
    (B) FILING DATE: 04-JUN-1996

(viii) ATTORNEY/AGENT INFORMATION:
    (A) NAME: Olson, Arne M
    (B) REGISTRATION NUMBER: 30,203
    (C) REFERENCE/DOCKET NUMBER: TSRI540.1PCT

(ix) TELECOMMUNICATION INFORMATION:
    (A) TELEPHONE: 312-580-1180
    (B) TELEFAX: 312-580-1189

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 2793 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: double
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGATCCCTGC TCCAGCAGCT GCAAGGTGCA AGAAGAAAGA GATCCCAGGG AGGAAAATGT 60
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TGCTATCCCA GAAGTCCGCC GATGACACCA AAACCCTCAT CAAGACCATG TGCACCAGGA 180
TCAATGACAT TTCACACAGG CAGTCGGTAT CGGCAAAAGCA GAGGCTGACT GCTTGGAGCT 240
TCATTCCTGG GCTTCACCCC ATTCGAGAGGT TGTCGAAGAT GGACCAGACT CGGCGAGTCT 300
ATCAACAGGT CCTCCAGCACG GCTGTCCCTCC AAAATGTGCT GCAGATAGCC AATGACCTGG 360
AGAATTCTCC AGAACCTCCCT CATCTGCTGG CTTCTCCCAA GAGCTGCTCC TGGGCTCAGA 420
CCAGTGCCCT CGCAAGGCAAG GAGAGCCTGT GAGGCTGCTCT GGAAGCTCCA CTGCTTTCCA 480
CAGAAATGCGT GTCTTTGAGG AGCTGCTGCA GCTTCTGCA GGACATTCTT CCAGATGTTG 540
ATGTTAGCCC TGAATATGGGA AGTTTAAAAA AACTCCAGGG TCCCAAGGAAT CTTGTTAGGG 600
GAAAGAAACTT TGGCTCCAGG GGTTCTCAG GAGAAGAGAG CCATGTCGAC ACATCCATCA 660
TTCATTTTCTC TCCGTCCCTG AGACCCACGCA TCCAAAGCACT TGACTCCACA ATGCTTTGACT 720
CAAGTTATCC ACAACACTTG TGAAGCAGAA GAGAGGGCCA GCTTGCAAGAG GGGACTCTCA 780
CCTATGTCCTT CAGAAGTAGA AGATAAGAGC CATCCCATCC CTTCCATGTC CCACCTCTTC 840
CGGTATACATG TTCTCCCTTG GGTACACGCT TCGCTTCGCC CCAGGAGAGG TGGATTAGGG 900
ATGGTGTAAG CTTTGGGCT GTCTCAGAGT CTTTGGGAGGC ACCGTAAGAG CTGCACTCAGC 960
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ACTCTTTCCG GAACATTTTTT AAGTGCTCAGG CACCCCTTGG AGGGCTAAA GCTACAGGCC 1560
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CAAGAAAGGG GTCCCTGGGT TAGATCTCCA AGGTTGATCG CAAATGCGGT 1680
(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 3862 base pairs
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: double
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
    (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTCGACCCAC GGCTCCGGAG GAATCGTCTC GCAAAATCCAG GCTGACACCT CTTACACACC
AGATGGTGGT GCTGAGATGC TCAGATTTCA GTCGACTCTG ATGCTTCGTG

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GACCTTAACC TGGCATATCC AATCTCTCCC TGGAATTATA AGTTGGTTTTG TGGACCACCG 180
AACACAAACG AGTACTCTTT TCTCTCACC GTCTGGAGCCC CAAACAAATGC CTGGGCTTTTG 240
AAGGGGCTTT CTGGAAGGATG TTGGTAAAGCT AAATTTAATT CAAGTGTTATG CTACGTTTCT 300
GAGTTTTCCAA AAACAGCTTT CCAACTTGGC TTGGAGGAGT AGCAGAGTTCA AAACCTGCCT 360
GCACTCAGAG ACAAACACTGA AGGGAAGACA CGTGGCTTCA TATGGAAAGGC TCCAGTTCTT 420
CGCCAGCTAG GTGTAAAGTG GGCATAGAG TGCTGGATGA AAGGGGACTT GACATTATTAC 480
ATCTGTGACTT GGGGCCATTT ACCCTCAAGAC ATTATGACTC TAAGGTCCAT 540
CTTTTATATG ATCTGGCTGA AGTCAAGTATG GATTCGCTCT TGCCCCACTGG AAAGAGACC 600
TTCCAGACTG TTGCAATGCAA CTTCAGTCTT CGGGGGATGTG AATGTCATTG GCCGGTACC 660
AGGAGCAAAC CAAACTACGC TTCTCTGATG TATTTTGGAA TCACTACRTC CGTGTTGAGT 720
TTCCAGTCTC CATCCTGATTC ACGTCAAGGCC ATCGTCTTTG TGAAGGCCAG GGCACCTCTT 780
GTTTTGGGATG GGGGAATCTC AGATGGATGTG AAATTAAGAG TTTCTTGGGA CAGCCAAACA 840
ATGGGACCAG TGGCGCTTCA ATACAGGTTG AATAATTTAG AGAATTCTAC AATGTGAAAG 900
GAGGCTGCTG CAAATGCTCT ACACTCAGCT CTGTCTGTAG ACGTGTGTCT TCCTGTGATC 960
TCATATAGGG TCGAGCTGGAG GACCAAGGAG CTGGAATGTTG CAGGTAGCTG 1020
AGTTCACCTC AAGTCTTTAC CACCAAAGAT GTTGTGATTT TTTCCACCAC AAATTCGACT 1080
AGTGGTGGAG TAGATGCTTC TTTCATGTC ATCTCAAAAA AGAAACCCA GATTATCTCC 1140
TCAAAACAGA TAGGTGCTTT GGGAAATCTA GCTGGAAAAA TCCCTGAGAT AAGTGACAGC 1200
ATTTGTGATTG ACCGGAGTGTG AAAAGCTACCC TCTTCCTGCA TGAAGCACC CAGACCTCAG 1260
GGGGAGTTTA CCTATGACGC AGTGTACTGC TGCAATGAGC AGGCGGTGCA TCAAGCTATG 1320
GC TGAAATGT AGTGTATCGA TGTCATTATC AATATATCAT GTGAAACTGA CGGGATCTTA 1380
ACTAAAATGT CTGCGAGATG GTGCCCAGCC ACAAATCCTA CACTGTTGGG AGCACTCTG 1440
CAGCTGAGGT ATCACAAGGCG CAGCCTGTATG TTGGCGTATA GCACATATCT TACATCTCAG 1500
TCTGAGCCCC AAAACTGCGT CTCTACACAG GAGGGGCTTTT ATGGATGTTT TTTCCAGCCA 1560
ACTCTTCTAT TCTTGGTATA CACAATGTTG ACAGGATGCA ACCATTCTT CAGTTACCTT 1620
GACCTGCCAC CAAGTGTGTG CCTTCGTGAC TCCGTAATGA AACCACATTC TCCATCTAAC 1680
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TGGAAGACAC ATGAGGATTT CGCTGCAAAAG TCAAGAATCTG CCAGCCGTCT GGTGTCGAC 1860
CTCTGACGCG TCTATCTGGGT CAGGGTCTGC TGCCGGGCGG TGAGTGAGACT AGGATATTTG 1920
AGTAATTGGA GCAGTCCAGC ATATCCGCTT GTCTAGGATG TAAAGGTTCC TATGAGAGGG 1980
TGAAAAAAA AAAAAAAA AG

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1974 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AAGTCTCCAG GGCAGAGGG GAGTCACCTC ATTTGCGGCTT GAGTCGGCAA AGAAATCAAG
ATGGCCAAAG TTCTCTGCTT GGGTGAAGAC CTAAGAAGCT GTTACAGTGA AAACGAAGAC
TACAGGGTCC CCATTCACCA CTCTCTCTTG AATCAGAATAT CTTCTCTAGA TGCAAGCTAT
GGCCTCCCTTC ATGAGACTTG CACAGATCGT TTTGATATTCG TGAAGACCTC TGAAAGCTCA
AAGATATCCTA AACTTCACCTT CAGGAGAGCG CCGGGAGCAAG TATCAGAACC GTCAAGGAAC
GGGCAAGTAGT AAGAAGAGAG TGGAGGTGAG TACAGTGAGA CACTTCAGAG ATGACCTCTG
CAGTCATTAA CCAAAGGACTCC ATTTATCCCA GATCAACCCCG TACAGACACC TTACACCTAC
CAGATGTTTT CAGAGTACAA ACTGAGAAGGT CTCGTCAGGC AGAAGGTTGT CATGAATGAT
TCCCTCAACC AAACATATA TCAGGAGTGTG GACAACACT ATCTCAGACG CACTTGTTTA
AATGACCCGTG AACAGGAAGTT AAAATGGACTGTAAGTATGCGGG ACTCGTGCGG AGGAGAGAG
TCTAAATATCC GTGTTACTCT AAAAACTTTCA GATCCACAAAT TGGTTGAGG CGCTCAAGGA
GAAGACCGAG CCGTGGTGCT GAAGAGAGTG CCAGAAACAC CAAAATCTCA CACAGGATGT
GAGACCGACC TCATTTTCCTT CTGGAAGAGG ATCAACTCTTA AGAAGACTTTT CACATCAGCT
GCTTATCCAG AGTGTGGGAT TGGACACAAA GAACAAAGTG GGGAGCGCC CCGCGGGAG
CTGCCCTTCTA TACAGAGCTC CCAGATATCAT AAAAAAGCGCC TTATTTGCGC GAGTCTATTC
ACTTGGGAAG TGCTGACTGT CTGTATGTAC CATGTACAGG AACCTCTCTC ACCCTGAGT
ACTTGCACAG CATGTGTCTGA GTCTCTGTAA TTCTTAATGA ATGTTTACCC TCTTTGTAAG
AGAAGAGCAA ACCCTAGGG AGCCACCCCC ACATATGATA CTATCTGTTA TTATTTAAGAG
TACCTTATAG TTTGCTCAAG ACTAATACATT TTAATTACATA TTCTGATAGG CATCTCTAGA
AGGATCAAATA AGACTCTACA CATATTACAG ATGGGTTAAC AAAGGATAA ACAACTGGA

3862

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AAGCACACTC AATGCATTGG GAATATAAAT TCACAGACCA ATCTCAGCTGT GCACCTTCCGG 1260
CTCAAAAATG CCAGGGAGT AGGATAAAGG TATAAGAACT TAATGCTGTC ATTITCCAAA 1320
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TGGATGAAAT CACTCCGAA CAGACCCCAA GAAGGAGACA GACTGTTGAA TGTTTATTTTTT 1440
AGTATATTTT ATATATATAT TTAAATAATAT ATTATATAT ATATAATGATA 1500
TCCTAAAATC CTCTGAGCCTT GACAGGACAT CTCAAGACGAC GATTTTCTTAG GTGGTCAGTGT 1560
AGATATAGTT TTCTCTAGAC CAAATGCTTA CACACTTTTAC ACTTTTTCCAG CAGCCACGGAA 1620
GCTCTCTGTA CATTCCTGTA CTTGCGGCC CTTTCATCAT GATCTTAATC TGCTACGTATT 1680
ACTTTGTTCG TCTAAAATGGA TAAATCGAGTC AGTCTTTTTT CCTCCCATCC TTAAGAAGCTGT 1740
CTGGGTATTG TTACATCGAT CAGTCCTCAG TTTAATCTAAC ACCAACATTCC TAAAGATGGA 1800
AAGAGCTTAA CTCTGACACA CACATCAGCT AGTACGAAAG GTTTTTTTCT AGAATGTAAT 1860
CAGTGTTCGCC CAGGACCTT AATTTTTTTTT TCAAAACCACA GTATCATGTA ACATACCAAC 1920
ATAACAAATCA ACTCATTATT ATATATCATA ATTAATATAA ACAAGTTTGA GCTG 1974

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1339 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
TGCAGGGTTC GAGGCTCTAT AGGCCTACCT GTGACGCTCTT CCAAGCAAGC TTTCTTTGTCG 60
AAGGTCTCGA AGCAGCTGAT GCAACTGTTG CTTAGACTCAA CTGTAATAAGT CCCACCTTTTG 120
ACAGTGTAAG GAAATGACCTT CTTCTTGAGG TTAGCGGGGCA CCAAAGAGCT AAGGCGCTGT 180
TCCAAACCTT TGACTGAGTG GTGCAGGATTG AGAGCATCCA GCTTCAAATC TCACAGCAGG 240
ACATCAAAAC CAGCTGACGG CAGGACATAT CACTCATTGT GGCTGTTGGA AGGCTGTGGC 300
AGCTACCTGT GTCTTTTCGG TGGACCTTCC AGAGATTGAG GATGAGCCAC TCTTTTTCCT 360
TCATCTTCAG AGAAAGAGCCC ATCCTCTGTG TACTGTGGGA TGATGATGAT AACCTGCTGG 420
TGTGTGACTG TCCATTGAGA CAGCTGACTC AGAGGCTCCG AGATGAACAA CAAAAAGGCC 480
TCGCTGCTGTGACCCCGATATACCTCAAGGACGACCTCAATGACACCAATGACTCAACC
AAACAACTGATATTCCTCAGTACGTTAATACCCAAAGGAAAGACCAAAATACCTG
TGAGCTGGCGCTAAGGAGAGAGAGTACCACTCTGTGTAAGAAAAGACGCGACAC
CCACCTGCTGCACGAGAAGGATCTCCGGACTAACGTACCAGAATAGATGGG
GGTTTGTCTTCAGAGTTCTCAAGGACAGGTTTGAATTGCTGATTAGTGGCCAG
CCTCAGGCACTCAACGCGACGGCAATTGCTGTCGATCGTTCAGAAGATACG
GTTCGATCAAGAACGTGACCTCTCACCATTTAATGATATGAAAGAAGCTGG
ACGTTTCTAAAATGAGGATTCTGCTGAGTCAAGAGG GCCGTCGAC
AGCTCAATCTAGGAACTTCTAGCCTTAGTTTCTCAGCCCAAGAACGGTTCAAG
AAACCATGGCCAGGTTTTGCTGAAGAGAGACCTGCTGTTTCTCAGGTTTCAG
CCACTTACCTGATTATGTATGATGATCTACTTGAGATTGAGATGATTCAG
GGCCATAGGGCAGACCATTTGCTACGACACGGCGAGAGAGATTAGGAGAGAAG
GTGCTGCTGGAGAAGGGCTGAGGAAATAAGATGGTTCTTATTTAAATCTAAG
TGGATGAGGAAAGGTCGTGCTGAGGAAGGTGAGTTATGAGTGGATGATGAGGG

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1629 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCTGAGGGAC TAGAGCAGGGAGGAAAGACGAAATATGCAAGAATAGCTCCCA
AAAAGCAACGAGCCAGGGTCCTCTGGGCAACTTCGCTTTCCTGACTGCAAGC
CATCTCCCTCATGAAGAAACGACCATTGAGGAGAGCAGACGGCAGGAGGGACTGG
CCAGAGAGGGCTCCTCCACCAAGAGATGGGGGTGCTCCAGGACCTCCAGGCGAT
TCAGCTTTCTGTGATCAAGCAGGGCCACCCACGCTCCAGTCGGTCACAGCTT
TCGGGGTCTAGCATCCCAAGGCTAGAGAAGATCCCTTCTCTGTCACCTCAGT

60 120 180 240 300 360
CTATGGCCCA GACCCTCACA CTCAGATCAT CTTCTCTAAA TTCGAGTGCAG AAGCCCTGTAG 420
CCCACGTTCGT AGCACAACCAC CAAGTGGAGG AGCAGCTGGA GTGGCTGAGC CAGGCCGCCA 480
ACGCCCTCCCT GGCCAAGGCG ATGGATCTCA AAGACACACCA ACTAGTGCTG CGACGCCGATG 540
GGTTGTACCT TGTCCTACCT CAGGGTCTCT TCAAGGGACA AGGCTGCCCC GACTACGTGC 600
TCTCACCACA CACGGTCAGC CGATTTGCTA TCTCATACCA GGAGAAAGTC AACCTCCTCT 660
CTGCCGTCRA GACGCCCTSC CCCCAGGACA CCCCTGAGGG GGCTGAGCTC AAACCCTGGT 720
ATGAGCCCCCC ATACCTGGGA GGAGCTCTCC AGCTGGAGAA GGGGGACCAA CTCAGCGCTG 780
AGGCTAAACTG GCCCAAGTAC TTAGACTTTG CGGAAGTCGG GCAGGTCTCA TTTAGGAGTC 840
TGTGCTCTGTG AAGGAATGG GTGTTCACTCC ATTCTCTACC CAGCCCCAC ACCTGACCCT 900
TTACTCTGAC CCCCATTATG TCTACTCCCT AAGAGCCCCA GTCTGTGCCA TTCTRACTTA 960
GAAAAGGATG TATGGGCTCAG AGTCTAACCT TGTGCTCAGA GCTTTCAACA ACTACTCAGA 1020
AACACCAAGAT GCTGGGACAG TGAGCTGGAC TGTTGGGCTC TCATGACCAC CCACCAAGAG 1080
AATCGAGAAA GAGCTATCAA TCTGGAAATTC ACTGAGACCT CAAATGTCCTA TTCTGTAGTT 1140
CTGCAAAGGG AGAGTGTCGA GTGGCCCTCT GTCTCAGAAAT GAGGCTGGAT AACATCTCAG 1200
GCCTCTCTAC CTTTCAACTC TTTTAGGAGG TGGAGTTAGCC AGCTGCTGCA ACTGCCTCCC 1260
ACAGAGCCAG CCCCCCTCTA TTATATTTTG CACTTATTAT TTATTATTTA TTATATTTAT 1320
ATTTATTTGC TTATGAAATGT ATTATTTTGG AAGGCGGGGG TGCTCTGGAG GACCCAGTGT 1380
GGGAAGCTGT CTTTACAGAG ACAGTTTTTC TGAAAACGG GAGCTGAGCT GTCCCCACCT 1440
GGCCTCTCTA CTTTGTTGCC TCCCTTTTTG CTTATTTTTA AAACAAAAAT TTTACGTACAC 1500
CCAAATTGTCT TAATACGGC GTATTGGGTA CCAGGCTGTAC GCTACATCA CAGACCTCTGT 1560
CTCCCAAGGG GAGCCGTGCAG TGTAATTGCC CTCAGGTTCA TTGGAGAAGA TAAAGATCGC 1620
TTGGAAAAG 1629

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
   (A) LENGTH: 4110 base pairs
   (B) TYPE: nucleic acid
   (C) STRANDEDNESS: double
   (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
   (A) ORGANISM: Mus musculus
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GAGACTCTGG CCCCACGGGA CACAGTGTCGA CTGTTTGGAA ACTTCTCAGC CACCTTGGTG 60
AAGGGACTGA GCTGTTAGAG AACTTCTCGA GGCTCTCACG GCTGGGCTCT TGGTACTCC 120
ACGGAGTGAC CTAGTCAACT CGAAGAGAAC AGAGAAGGGT GATTGTGGAG CAGAGAGTCA 180
AAGTGTCAGA CATGGTTCGG CCCCCTGAAAT TCTCTCTCAA AGTCAAAATGG TACCAAGTGG 240
ACCTGAAAGA GAGAAGGGAA ATAAAACAC AGCTGAAGAA AACCCTCTGT GCTGTCTTCA 300
GCCCAACCAT ACAAGATGAC CCTAAGAGTC ACCAAAATGG CTCCCAGCAG CTCTCTACTG 360
GCCACAGCA AATGTCTTCA GAATCTCTTG ACAAGCTGCA TGTTACATCG ACCGCTGCCA 420
AGTATGCTAG GATCAAAAGA TTGGCGCTTG GAGAGATTGT GCAATGACTG CTTCACCAAC 480
AGGCCACATC GGATTTCTAG TCGAGTCTCA AGTCTTGGTT GGATTGGTTC ATGAAACCCA 540
AGATTTTGC CAGAGCTACC AGAGAAGACC CTACCCTCTT GAGAGAGTCA CTGCTCTCGT 600
CCATGAGATT CATCAACACC TATATGGCT CTTTAAAAGA GCGAAAAATA GAGGAACATC 660
TGCGCCAGCT GGAAGCTGTA ACAAAGGAAA TACAACAACAG AGAAGCTTAC CAGCTCAGCT 720
TGGATAGCTCATCTTGGCC ACCAAGATGG CCTGGAGGAA TGCTCCTGCA TCAGACGGCA 780
GATCCAGTGG GTTCCAACCTG CAGTCTTTTT AGCTCTGGGA ATGTAGCCAC GAGAAGAAA 840
TGATTCGACC CATCTGCGCA CACATCTTTT ATGGCCACCA CAATGGAAC ACAGAGCTGG 900
CCATCAGCTG GTCTCCCCAG CAGAGTGACG GAAACACTGA CTTCTAGCTG AGAAATACAC 960
AGCTCATCCG GTACGCTGGC TACCAAGATGC CAGATGAGAC ACAGAGAGGG AGATGCTGCCA 1020
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CTGATCTTGT GATGCCAGTG ACCATGACG ACATCCAGTA CCAGTGGTGC CAGAAGCTCG 1200
GGTTGAGATG GTATGCAGCTG CCTGCGTGGG CCACACATGT ACTGGAGGTT GGTGGCTTCG 1260
AATGCCACGC CTGCCCTCTC AATGGTGGAT CAGATGCGCA AGAAGATGGA TGGAGACTG 1320
TCTGTGACAC ACAGCAGCTAC AACACACTGAG AGGAAGTTGG CCGAAGGATG GCCGGCTGGA 1380
CCACACACTG GCTCTCCCTG TGGAAAGACC GGCTCTGACG GAGATCAAT GTGGCTCTGAG 1440
TCCATAGTTT CCAGAAGCAG AAGTGCAACA TCAAGAACCA CCCACAGCC TCAGACCTCT 1500
TCATGAAGCA CATGCAAGAT GAGTACCGGG CCCCCTGGAGG CTCCCAGGCA GACAGGATTT 1560
GGTCGCTCC CTCAGTGCTC GGAGACATCA CCCCTTGTGT CCACAGAGG ATGTTGACTC 1620
ATGTCTCATT TCCATTCTAC TACTACCAAG TGGAGCCTTG GAGACCCAC ATCTGGCAGA 1680
ATGAGAAGCTT GAGGCCAGG AGAGAGAGGA TCGATTAG AGTCTTGGTG AAAGGTGCTGT 1740
TCTTTGCTCTC CATGCTAATG CGAAGAGTGA TGCCCTTACG GGTAGACGCC ACAGTGCTCT 1800
TTGCTACTGA GACAGGGAAG TCTGAAACAC TACGCCAGGA CTTGGCCACC TTGGTCAGCT 1860
ACGCTTCAA CACCAAGGTT GTCTGATGAG ACCAGTATAA GGCAAGCACC TGGGAAGAGG 1920
AGCAACTACT GTGGTGGTGAG ACGAGCATAT TGGGGAATGG AGAATTTGCCC 1980
AGACTGAGAA GAAAATCTCTG TCTAGCTTAA GAGAAGACTCA CCACACCCCTC AGGTAGGTCTG 2040
TGTTTGCCCT TGGCTCCAGC ATGTACCCCT AGTTTCTGCG CTTTGTGTAT GACATCGGAC 2100
AGAAGCTGTC CCACCTGGGA GCCCTCTGAC CTTGCCCAAC AGGAGAAGGG GACGAACTCA 2160
GTGGGCAGGA GGAATGCTTCC CGCACTGGG GCTGAAACAAC CTTCCGGGCA GCCTGTGAGA 2220
CCTTTGATGT CGGAGAGCAA CATCACATTG AGATAGGGAA AGCCTTCATT CCAAATGCAGA 2280
CATGGGAGCC ACAGCAATAT AGGCTCATCC AGAGGCGGGA GCCTTTAGAC CTCACAGAGA 2340
CCCTCAGCAG CATCCCATCA AAGAAGGCTG TTACCATAAG GCTGAAATCC CAGCAAGATC 2400
TGACAGACGA AAGTGCCAGC CGCAGCACCAC TCCTCTGCTCA GCTCAGACTT CGAGGCGGGC 2460
GAGGGCCCAG CTACCTGCTG GGGAAGACCC TGGGGATCTT CCCAGGCACA CAGACCGCCC 2520
TGCTGACGAG AAATCTTGGG CGAGTTGCTT AGTTGTCTAC ACCACACCAA ACTGTGTGCCC 2580
TGAGGTCTTC GGAATGGAGC GGCAGCTTCT GGTGCAAGAG CAGAGGCTGC CCCCCTCCTG 2640
CAGTCGGCAG AGCCCTCACC TACTGGATGC ACATTACAGC CCCCTCCCAG CAGCTGGGAC 2700
TCCACAAGCT GGCTCGCTTT GGCAAGGAAG AGACGGAATG GCAGAGAATG GAGGCTTTGTT 2760
GTCAGCCCTC AGAGTCAATA GACTGAAAGT TCAGCAACAA CCCACCGTTC TGGAAGGTGC 2820
TTGAAGAGTT CCCCTCTTCT CATGATGCGGC CTGGATCTCT GCTGTCCGAC CTGCCCTATC 2880
TGAAGCCGCCT ATACCTACCC ATCAAGCCTCT CCCAGGACCA CCACCCCTCG GGAGGTTACC 2940
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CAGGCTGACT CCGGGCTGCC GGGCACCACCAG AGGTCTACTT TCAGGACTGC CTCCAAAGAC 3360
AGCTGCCCAG TGAGGTACTT AGCGTCTCCT AGCGGACACA GGGCCAACCAG TACATTTGGG 3420
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TGAAACCTGAG CGAGGACAGC GTGGAGAAGTT ATTTCTTCCA GCTCAAGAGC CAGAAAGCTT 3540
ATCAAAGAGA TATCTGGGTC GCAGCTTTTT CCTATGGGGC AAAAAAGGCA AGCGGCTTGG 3600
AGAGCCGCAA AGCCAGGAGG CTCTGACAGC CCAGGAGTTCC AGCTTCCTGCG ACTGAGTAAA 3660
GATAATGTTG AGGGGCTTGG GGAGACAGCG AAATGCAATC CCCCCCAAGC CCCCCATGGT 3720
ATTCCCCCCT CCTCCACCCC ACCAAGTATT ATTGTATTAT TGTTGACTAT TAATACTCTC 3780
TCCTCTCTCT CCTCCCCCTCT TCCCCCTCTC TCCACTCCCC AGCTCCCCCT 3840
TTCTCTTTCT CCTCTTTGAC CTCTCACCTC TTCTGGAAGC TGAGAGAGAGA GAAAAACTCA 3900
ACCTCTGAC TGAAGCAGTT TGGTGGAACCC CACCGGAGCA CCAATGGGCCC GCTCTAATAA 3960
TTAGCTGCAC TAGTTCACAGA TATTATTACT TCATATATTA GAAACAGAT ACCTTTGTCT 4020
ACTCCCAATG ATGGCCTTGG CCTTTCCCTG ATAAATTCCCT GATGAAAAAT ATTTTATATA 4080
AATACATTTC ATTTAATCA AAAAAAAAAA 4110

(2) INFORMATION FOR SEQ ID NO:7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 465 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHEtical: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Rattus norvegicus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
GGCATCATGG CGCCCCTTCCGG CCTCTCTGGTG AAGCCCAAGA TCGTCAAAAA GAGGACCAAG 60
AAGTTCCATCA GGCACCCAGTC GGACCGATAT GTGAAAAATA AGCGAAACTG GCCGAAAACC 120
AGAGGCATCG ACAACAGGGT GGCGAGGAAGA TTCAAGGGCC AGATCTGTAT GCCCAACATT 180
GGTTACGCGA GTAACAGAAAG AACCAAGCAG ATGGCTCCCTA GGCGCTCCG CAGGTTTCTG 240
GTCCACAAATG TCAAGGGCTG GGAAGTGTCTG CTGTGTGTGCA ACAAAATCTA CTGTGTGTGA 300
ATTGCTCAACA ATGGTCTCCCT TAAAGACCGA AAAGCCATCC TAGAAAGAGCC AGCAGAGCGT 360
GCCATCAGAG TCACCAATCC CAACGCCCCG CTACGCAAGCG AGAGAATGA ATAGATGGCT 420
TGTGTGCTTG TTTTGTGTGC AAATAAAAACC ACAAACACTG CAAA 465

(2) INFORMATION FOR SEQ ID NO:8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GCTATCGACA AGACAGCAGAA T
21

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
TGAACACAAC AACATAAAGC CC
22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
TGTTATATCT GGTTATTATT GAATGG
26

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
    (A) ORGANISM: Mus musculus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
    CATTAATGA TTTATTATCA GAATTGC
WE CLAIM:

1. A method for treating a patient having a condition in which regulating energy metabolism during a systemic inflammatory response is desired, comprising administering a composition having a physiologically effective amount of at least one OB-R agonist ligand.

2. The method of claim 1 wherein the OB-R agonist ligand is recombinant human OB protein.

3. The method of claim 2 wherein the amount of recombinant human OB protein administered is from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

4. The method of claim 1 wherein the OB-R agonist ligand is a peptide conformational analog of human OB protein comprising conservative substitutions of amino acid residues.

5. The method of claim 1 wherein the OB-R agonist ligand is an OB-related peptide.

6. The method of claim 1 wherein the condition is sepsis.

7. The method of claim 1 wherein the condition is systemic inflammatory response syndrome.

8. A composition for treating a patient having a condition in which regulating energy metabolism during a systemic inflammatory response is desired, comprising a physiologically effective amount of at least one OB-R agonist ligand.

9. The composition of claim 8 wherein the OB-R agonist ligand is recombinant human OB protein.

10. The composition of claim 9 wherein the amount of recombinant human OB protein per dose is from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.
11. The composition of claim 8 wherein the OB-R agonist ligand is a peptide conformational analog of human OB protein comprising conservative substitutions of amino acid residues.

12. The composition of claim 8 wherein the OB-R agonist ligand is an OB-related peptide.

13. The composition of claim 8 wherein the condition is sepsis.

14. The composition of claim 8 wherein the condition is systemic inflammatory response syndrome.

15. A composition for the amelioration of the toxicity of therapeutic cytokines comprising a physiologically effective amount of an OB-R agonist ligand.

16. The composition of claim 15 wherein the OB-R agonist ligand is recombinant human OB protein.

17. The composition of claim 15 wherein the amount of recombinant human OB protein per dose is 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

18. A method for the treatment of a patient having obesity comprising the steps of:

   administering at least one OB-R expression inducer; and

   administering a physiologically effective amount of an OB-R agonist ligand.

19. The method of claim 18 wherein the OB-R expression inducer is a compound chosen from the group consisting of LPS, IL-1α, IL-1β, TNF-α and IL-6.

20. The method of claim 18 wherein the OB-R expression inducer and the OB-R agonist ligand are administered at a different times.

21. The method of claim 18 wherein the OB-R expression inducer is administered in an amount from about 0.003 to about 20 micrograms per kilogram body weight.
22. The method of claim 18 wherein the OB-R agonist ligand is administered in an amount from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

23. The method of claim 18 wherein the OB-R agonist ligand is recombinant human OB protein.

24. The method of claim 23 wherein the recombinant human OB protein is administered in an amount from about 1 micrograms per kilogram body weight to about 50 micrograms per kilogram body weight.

25. The method of claim 18 wherein the OB-R expression inducer is IL-6.

26. The method of claim 25 wherein IL-6 is administered in an amount from about 0.5 to about 20 micrograms per kilogram body weight.

27. A method for the treatment of a patient having a condition characterized by OB resistance, comprising the steps of:

   administering IL-6 in an amount from about 0.5 to about 20 micrograms per kilogram body weight; and

   administering recombinant human OB protein in an amount from about 1 microgram per kilogram body weight to about 50 micrograms per kilogram body weight.

28. A composition suitable for the treatment of obesity comprising:

   at least one therapeutic cytokine capable of increasing the expression of the OB receptor;

   a physiologically effective amount of an OB-R agonist ligand; and a pharmaceutically acceptable excipient.

29. The composition of claim 28 wherein the therapeutic cytokine capable of increasing the expression of the OB receptor and the OB-R agonist ligand are packaged separately.

30. The composition of claim 28 wherein the therapeutic cytokine is about 0.5 to about 20 micrograms per kilogram body weight IL-6.
31. The composition of claim 29 wherein the OB-R agonist ligand is administered in a dose of about 1 micrograms per kilogram body weight to about 50 micrograms per kilogram body weight recombinant human OB protein.

32. An assay kit for a disease marker in a sample for a systemic inflammatory response in a patient comprising:
   - an antibody capable of binding to OB protein; and
   - a detection means for determining the amount of the antibody bound to OB protein.

33. A method for assaying a disease marker for an inflammatory response in a patient comprising:
   - mixing a portion of the sample with an antibody capable of binding to OB protein; and
   - detecting the amount of antibody bound to OB protein.

34. A composition suitable for the treatment of anorexia, cachexia or other wasting condition comprising a physiologically effective amount of antibody capable of binding OB protein.

35. The method for the treatment of anorexia, cachexia or other wasting condition comprising administering a physiologically effective amount of antibody capable of binding OB protein in an amount from about 0.02 to about 15 milligrams per kilogram body weight per day.
FIGURE 2
FIGURE 3
FIGURE 6
Body Weight (g)

- Vehicle
- Anti-OB

Days

0 1 2 3 4 5 6 7

FIGURE 9
FIGURE 10
FIGURE 11
FIGURE 12
FIGURE 13

Graph showing body temperature (°C) over hours with control and mOB lines.
FIGURE 15
FIGURE 16
# INTERNATIONAL SEARCH REPORT

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 38/00, 45/05, 38/19, 39/395  
US CL : 514/2, 8, 12; 424/85.1,158.1, 145.1  
According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

- U.S. : 514/2, 8, 12; 424/85.1,158.1, 145.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

- CAS ONLINE, DIALOG, MEDLINE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tbody>
<tr>
<td>X</td>
<td>WO 96/05309 A3 (THE ROCKEFELLER UNIVERSITY) 22 February 1996, (22-02-96), see the claims.</td>
<td>1-35</td>
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<tr>
<td>X, P</td>
<td>WO 96/35787 A1 (CHIRON CORPORATION) 14 November 1996 (14-11-96), see entire document, and claims.</td>
<td>1-35</td>
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<tr>
<td>X</td>
<td>HALAAS et al. Weight-Reducing Effects on the Plasma Protein Encoded by the obese Gene. Science, 28 July 1995, Vol. 269, see pages 543--547.</td>
<td>8-17, 18-26</td>
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Further documents are listed in the continuation of Box C.  

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Date of the actual completion of the international search: 02 SEPTEMBER 1997  
Date of mailing of the international search report: 23 SEP 1997

Name and mailing address of the ISA/US Commissioner of Patents and Trademarks  
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Telephone No. (703) 308-0196
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<tbody>
<tr>
<td>X, P</td>
<td>SARRAF et al. Multiple Cytokines and Acute Inflammation Raise Mouse Leptin Levels: Potential Role in Inflammatory Anorexia. Journal of Experimental Medicine. 06 January 1997, Vol. 185, Number 1, see pages 171-175.</td>
<td>1-35</td>
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