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**WO 01/92474 A1**

(54) Title: ALPHA (2) MACROGLOBULIN RECEPTORS AS A HEAT SHOCK PROTEIN RECEPTOR AND USES THEREOF

(57) Abstract: The present invention relates to the use of alpha (2) macroglobulin (" $\alpha$ 2M") receptor as a heat shock protein receptor, cells that express the  $\alpha$ 2M receptor bound to an HSP, and antibodies and other molecules that bind the  $\alpha$ 2M receptor-HSP complex. The invention also relates to screening assays to identify compounds that interact with the  $\alpha$ 2M receptor, and modulate the interaction of the  $\alpha$ 2M receptor with its ligand, such as HSPs, and methods for using compositions comprising  $\alpha$ 2M-receptor sequences for the diagnosis and treatment of immune disorders, proliferative disorders, and infectious diseases.

5 **ALPHA (2) MACROGLOBULIN RECEPTOR AS A HEAT SHOCK PROTEIN**  
**RECEPTOR AND USES THEREOF**

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awarded by the National Institutes of Health. The government has certain rights in the  
invention.

### 1. INTRODUCTION

15 The present invention relates to the use of alpha (2) macroglobulin ("α2M") receptor  
as a heat shock protein receptor, cells that express the α2M receptor bound to an HSP, and  
antibodies and other molecules that bind the α2M receptor-HSP complex. The invention  
also relates to screening assays to identify compounds that modulate the interaction of an  
HSP with the α2M receptor, and methods for using compositions comprising α2M-receptor  
20 sequences for the diagnosis and treatment of immune disorders, proliferative disorders, and  
infectious diseases.

### 2. BACKGROUND OF THE INVENTION

#### 2.1. HEAT SHOCK PROTEINS

25 Heat shock proteins (HSPs), also referred to as stress proteins, were first identified  
as proteins synthesized by cells in response to heat shock. Hsps have classified into five  
families, based on molecular weight, Hsp100, Hsp90, Hsp70, Hsp60, and smHsp. Many  
members of these families were found subsequently to be induced in response to other  
stressful stimuli including nutrient deprivation, metabolic disruption, oxygen radicals, and  
30 infection with intracellular pathogens (see Welch, May 1993, Scientific American 56-64;  
Young, 1990, Annu. Rev. Immunol. 8:401-420; Craig, 1993, Science 260:1902-1903;  
Gething *et al.*, 1992, Nature 355:33-45; and Lindquist *et al.*, 1988, Annu. Rev. Genetics  
22:631-677).

35 Heat shock proteins are among the most highly conserved proteins in existence. For  
example, DnaK, the Hsp70 from *E. coli* has about 50% amino acid sequence identity with  
Hsp70 proteins from excoriates (Bardwell *et al.*, 1984, Proc. Natl. Acad. Sci. 81:848-852).

The Hsp60 and Hsp90 families also show similarly high levels of intra-family conservation (Hickey *et al.*, 1989, Mol. Cell. Biol. 9:2615-2626; Jindal, 1989, Mol. Cell. Biol. 9:2279-2283). In addition, it has been discovered that the Hsp60, Hsp70 and Hsp90 families are composed of proteins that are related to the stress proteins in sequence, for example, having  
5 greater than 35% amino acid identity, but whose expression levels are not altered by stress.

Studies on the cellular response to heat shock and other physiological stresses revealed that the HSPs are involved not only in cellular protection against these adverse conditions, but also in essential biochemical and immunological processes in unstressed cells. HSPs accomplish different kinds of chaperoning functions. For example, members  
10 of the Hsp70 family, located in the cell cytoplasm, nucleus, mitochondria, or endoplasmic reticulum (Lindquist *et al.*, 1988, Ann. Rev. Genetics 22:631-677), are involved in the presentation of antigens to the cells of the immune system, and are also involved in the transfer, folding and assembly of proteins in normal cells. HSPs are capable of binding proteins or peptides, and releasing the bound proteins or peptides in the presence of  
15 adenosine triphosphate (ATP) or low pH.

## 2.2. IMMUNOGENICITY OF HSP-PEPTIDE COMPLEXES

Srivastava *et al.* demonstrated immune response to methylcholanthrene-induced sarcomas of inbred mice (1988, Immunol. Today 9:78-83). In these studies, it was found  
20 that the molecules responsible for the individually distinct immunogenicity of these tumors were glycoproteins of 96kDa (gp96) and intracellular proteins of 84 to 86kDa (Srivastava *et al.*, 1986, Proc. Natl. Acad. Sci. USA 83:3407-3411; Ullrich *et al.*, 1986, Proc. Natl. Acad. Sci. USA 83:3121-3125). Immunization of mice with gp96 or p84/86 isolated from a particular tumor rendered the mice immune to that particular tumor, but not to antigenically  
25 distinct tumors. Isolation and characterization of genes encoding gp96 and p84/86 revealed significant homology between them, and showed that gp96 and p84/86 were, respectively, the endoplasmic reticular and cytosolic counterparts of the same heat shock proteins (Srivastava *et al.*, 1988, Immunogenetics 28:205-207; Srivastava *et al.*, 1991, Curr. Top. Microbiol. Immunol. 167:109-123). Further, Hsp70 was shown to elicit immunity to the  
30 tumor from which it was isolated but not to antigenically distinct tumors. However, Hsp70 depleted of peptides was found to lose its immunogenic activity (Udono and Srivastava, 1993, J. Exp. Med. 178:1391-1396). These observations suggested that the heat shock proteins are not immunogenic per se, but form noncovalent complexes with antigenic peptides, and the complexes can elicit specific immunity to the antigenic peptides  
35 (Srivastava, 1993, Adv. Cancer Res. 62:153-177; Udono *et al.*, 1994, J. Immunol., 152:5398-5403; Suto *et al.*, 1995, Science, 269:1585-1588).

Noncovalent complexes of HSPs and peptide, purified from cancer cells, can be used for the treatment and prevention of cancer and have been described in PCT publications WO 96/10411, dated April 11, 1996, and WO 97/10001, dated March 20, 1997 (U.S. Patent No. 5,750,119 issued April 12, 1998, and U.S. Patent No. 5,837,251 issued November 17, 1998, 5 respectively, each of which is incorporated by reference herein in its entirety). The isolation and purification of stress protein-peptide complexes has been described, for example, from pathogen-infected cells, and can be used for the treatment and prevention of infection caused by the pathogen, such as viruses, and other intracellular pathogens, including bacteria, protozoa, fungi and parasites (see, for example, PCT Publication WO 95/24923, dated 10 September 21, 1995). Immunogenic stress protein-peptide complexes can also be prepared by in vitro complexing of stress protein and antigenic peptides, and the uses of such complexes for the treatment and prevention of cancer and infectious diseases has been described in PCT publication WO 97/10000, dated March 20, 1997 (U.S. Patent No. 6,030,618 issued February 29, 2000. The use of stress protein-peptide complexes for 15 sensitizing antigen presenting cells in vitro for use in adoptive immunotherapy is described in PCT publication WO 97/10002, dated March 20, 1997 (see also U.S. Patent No. 5,985,270 issued November 16, 1999).

### 2.3. ALPHA (2) MACROGLOBULIN RECEPTOR

20 The alpha (2) macroglobulin receptor (herein referred to interchangeably as either “ $\alpha$ 2MR” or “the  $\alpha$ 2M receptor”), also known as LDL (low-density lipoprotein) receptor-Related Protein (“LRP”) or CD91, is primarily expressed in liver, brain and placenta. The  $\alpha$ 2M receptor is a member of the low density lipoprotein receptor family. The extracellular 25 domain of the human receptor comprises six 50-amino acid EGF repeats and 31 complement repeats of approximately 40-42 amino acids. The complement repeats are organized, from the amino to the carboxy-terminus, into clusters of 2, 8, 10 and 11 repeats, called Cluster I, II, III and IV (Herz *et al.*, 1988, EMBO J. 7:4119-4127). One study points to Cluster II (CI-II), which contains complement repeats 3-10 (CR3-10), as the major ligand binding portion of the receptor (Horn *et al.*, 1997, J. Biol. Chem. 272:13608-13613). The  $\alpha$ 2M receptor 30 plays a role in endocytosis of a diversity of ligands. In addition to  $\alpha$ 2M, other ligands of  $\alpha$ 2MR include lipoprotein complexes, lactoferrin, tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), and exotoxins. Thus, the  $\alpha$ 2M receptor plays roles in a variety of cellular processes, including endocytosis, antigen presentation, cholesterol regulation, ApoE-containing lipoprotein clearance, and chylomicron remnant 35 removal.

Human  $\alpha 2M$  is synthesized as a 1474 amino acid precursor, the first 23 of which function as a signal sequence that is cleaved to yield a 1451 amino acid mature protein (Kan et al., 1985, Proc. Natl. Acad. Sci. U.S.A. 82:2282-2286). In experiments with recombinant protein, the carboxy-terminal 138 amino acids of  $\alpha 2M$  (representing amino acids 1314-1451 of the mature protein) was found to bind the receptor. This domain has been called the RBD (receptor-binding domain; Salvesent *et al.*, 1992, FEBS Lett. 313:198-202; Holtet *et al.*, 1994, FEBS Lett. 344:242-246). An RBD variant (RBDv), a proteolytic fragment of  $\alpha 2M$  comprising an additional 15 amino terminal residues (representing amino acids 1314-1451 of the mature protein) binds to the receptor with almost the same affinity as  $\alpha 2M$ -proteinase (Holtet *et al.*, 1994, FEBS Lett. 344:242-246).

Alignment of  $\alpha 2MR$  ligands identifies a conserved domain present in the RBDs of  $\alpha$  macroglobulins. The conserved sequence spans amino acids 1366-1392 of human  $\alpha 2M$ . Conserved residues within this domain are Phe<sub>1366</sub>, Leu<sub>1369</sub>, Lys<sub>1370</sub>, Val<sub>1373</sub>, Lys<sub>1374</sub>, Glu<sub>1377</sub>, Val<sub>1382</sub>, Arg<sub>1384</sub> (Nielsen *et al.*, 1996, J. Biol. Chem. 271:12909-12912). Of these, Lys<sub>1370</sub> and Lys<sub>1374</sub> were shown to be critical for receptor binding (Nielsen *et al.*, 1996, J. Biol. Chem. 271:12909-12912).

Binding of ligands, including the binding to  $\alpha 2M$ , to  $\alpha 2MR$  is inhibited by  $\alpha 2MR$ -associated protein (RAP). RAP is a 39 kDa folding chaperone that resides in the endoplasmic reticulum and is required for the normal processing of  $\alpha 2MR$ . RAP has the ability to competitively inhibit the binding of all  $\alpha 2MR$  to all  $\alpha 2MR$  ligands tested. One study shows RAP to bind to complement repeats C5-C7 in cluster II (C1-II) of  $\alpha 2MR$  (Horn et al., 1997, J. Biol. Chem. 272:13608-13613); another shows RAP to bind to all two complement repeat-modules in C1-II except the C9-C10 module (Andersen *et al.*, J. Biol. Chem., Mar. 24, 2000, PMID: 10747921; published electronically ahead of print). Three structural domains, 1, 2 and 3, have been identified in RAP, consisting of amino acid residues 18-112, 113-218 and 219-323, respectively. Ligand competition titration of recombinant RAP domains indicates that determinants for the inhibition of test ligands reside in the C-terminal regions of domains 1 and 3 (Ellgaard *et al.*, 1997, Eur. J. Biochem. 244:544-51).

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#### 2.4. ANTIGEN PRESENTATION

Major histocompatibility complex (MHC) molecules present antigens on the cell surface of antigen-presenting cells. Cytotoxic T lymphocytes (CTLs) then recognize MHC molecules and their associated peptides and kill the target cell. Antigens are processed by two distinct antigen processing routes depending upon whether their origin is intracellular or extracellular. Intracellular or endogenous protein antigens, *i.e.*, antigens synthesized within the antigen-presenting cell, are presented by MHC class I (MHC I) molecules to CD8+

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cytotoxic T lymphocytes. On the other hand, extracellular or exogenously synthesized antigenic determinants are presented on the cell surface of "specialized" or "professional" APCs (macrophages, for example) by MHC class II molecules to CD4+ T cells (see, generally, Fundamental Immunology, W.E. Paul (ed.), New York: Raven Press, 1984). This compartmental segregation of antigen processing routes is important to prevent tissue destruction that could otherwise occur during an immune response as a result of shedding of neighboring cell MHC I antigens.

The heat shock protein gp96 chaperones a wide array of peptides, depending upon the source from which gp96 is isolated (for review, see Srivastava *et al.*, 1998, Immunity 8: 657-665). Tumor-derived gp96 carries tumor-antigenic peptides (Ishii *et al.*, 1999, J. Immunology 162:1303-1309); gp96 preparations from virus-infected cells carry viral epitopes (Suto and Srivastava, 1995, Science 269:1585-1588; Nieland *et al.*, 1998, Proc. Natl. Acad. Sci. USA 95:1800-1805), and gp96 preparations from cells transfected with model antigens such as ovalbumin or  $\beta$ -galactosidase are associated with the corresponding epitopes (Arnold *et al.*, 1995, J. Exp. Med. 182:885-889; Breloer *et al.*, 1998, Eur. J. Immunol. 28:1016-1021). The association of gp96 with peptides occurs *in vivo* (Menoret and Srivastava, 1999, Biochem. Biophys. Research Commun. 262:813-818). Gp96-peptide complexes, whether isolated from cells (Tamura *et al.*, 1997, Science 278:117-120), or reconstituted *in vitro* (Blachere *et al.*, 1997, J. Exp. Med. 186:1183-1406) are excellent immunogens and have been used extensively to elicit CD8+ T cell responses specific for the gp96-chaperoned antigenic peptides.

The capacity of gp96-peptide complexes to elicit an immune response is dependent upon the transfer of the peptide to MHC class I molecules of antigen-presenting cells (Suto and Srivastava, 1995, *supra*). Endogenously synthesized antigens chaperoned by gp96 in the endoplasmic reticulum [ER] can prime antigen-specific CD8+ T cells (or MHC I-restricted CTLs) *in vivo*; this priming of CD8+ T cells requires macrophages. However, the process whereby exogenously introduced gp96-peptide complexes elicit the antigen-specific CD8+ T cell response is not completely understood since there is no established pathway for the translocation of extracellular antigens into the class I presentation machinery. Yet antigenic peptides of extracellular origin associated with HSPs are somehow salvaged by macrophages, channeled into the endogenous pathway, and presented by MHC I molecules to be recognized by CD8+ lymphocytes (Suto and Srivastava, 1995, *supra*; Blachere *et al.*, 1997, J. Exp. Med. 186:1315-22).

Several models have been proposed to explain the delivery of extracellular peptides for antigen presentation. One proposal, known as the "direct transfer" model, suggests that HSP-chaperoned peptides are transferred to MHC I molecules on the cell surface of macrophages for presentation to CD8+ T lymphocytes. Another suggestion is that soluble

extracellular proteins can be trafficked to the cytosol via constitutive macropinocytosis in bone marrow-derived macrophages and dendritic cells (Norbury *et al.*, 1997, *Eur. J. Immunol.* 27:280-288). Yet another proposed mechanism is that HSPs are taken up by the MHC class I molecules of the macrophage, which stimulate the appropriate T cells  
5 (Srivastava *et al.*, 1994, *Immunogenetics* 39:93-98. Others have suggested that a novel intracellular trafficking pathway may be involved for the transport of peptides from the extracellular medium into the lumen of ER (Day *et al.*, 1997, *Proc. Natl. Acad. Sci.* 94:8064-8069; Nicchitta, 1998, *Curr. Opin. in Immunol.* 10:103-109). Further suggestions include the involvement of phagocytes which (a) possess an ill-defined pathway to shunt protein  
10 from the phagosome into the cytosol where it would enter the normal class I pathway; (b) digest ingested material in lysosomes and regurgitate peptides for loading on the surface to class I molecules (Bevan, 1995, *J. Exp. Med.* 182:639-41).

Still others have proposed a receptor-mediated pathway for the delivery of extracellular peptides to the cell surface of APCs for antigen presentation. In view of the  
15 extremely small quantity of gp96-chaperoned antigenic peptides required for immunization (Blachere *et al.*, 1997, *supra*), and the strict dependence of immunogenicity of gp96-peptide complexes on functional antigen presenting cells (APCs) (Udono *et al.*, 1994, *Proc. Natl. Acad. Sci. U.S.A.* 91:3077-3081), APCs had been proposed to possess receptors for gp96 (Srivastava *et al.*, 1994, *Immunogenetics* 39:93-98). Preliminary microscopic evidence  
20 consistent with such receptors has been recently obtained (Binder *et al.*, 1998, *Cell Stress & Chaperones* 3 (Supp.1):2.; Arnold-Schild *et al.*, 1999, *J. Immunol.* 162: 3757-3760; and Wassenberg *et al.*, 1999, *J. Cell Sci.* 1:12). One hypothesis is that the mannose receptor is used in the uptake of gp96, but no mechanism has been proposed for the non-glycosylated HSPs, such as Hsp70 (Ciupitu *et al.*, 1998, *J. Exp. Med.*, 187:685-691).

25 The identification and characterization of specific molecules involved in HSP-mediated antigen presentation of peptides could provide useful reagents and techniques for eliciting specific immunity by HSP and HSP-peptide complexes, and for developing novel diagnostic and therapeutic methods.

Citation or discussion of a reference herein shall not be construed as an admission  
30 that such is prior art to the present invention.

### 3. SUMMARY OF THE INVENTION

The present invention relates to compositions and methods for the use of the alpha (2) macroglobulin ("α2M") receptor as a heat shock protein receptor. The invention is based, in  
35 part, on the Applicant's discovery that the α2M receptor is a cell surface receptor for heat shock proteins. In particular, the Applicant has shown that the heat shock protein gp96,

hsp90, hsp70, and calreticulin binds directly to the  $\alpha$ 2M receptor, and that  $\alpha$ 2M inhibits re-  
presentation of gp96, hsp90, hsp70, and calreticulin-chaperoned antigenic peptides by  
macrophages. Because no precedent exists for receptors that recognize abundant and  
intracellular proteins like HSPs, the discovery of an HSP cell surface receptor was highly  
5 unexpected.

The present invention provides compositions comprising complexes of HSPs and the  
 $\alpha$ 2M receptor, and antibodies and other molecules that bind the HSP- $\alpha$ 2M receptor complex.  
The invention also encompasses methods for the use of the  $\alpha$ 2M receptor as a heat shock  
protein receptor, including methods for screening for compounds that modulate the  
10 interaction of HSP and the  $\alpha$ 2M receptor, and methods for treatment and detection of HSP-  
 $\alpha$ 2M receptor-mediated processes and HSP- $\alpha$ 2M receptor-related disorders and conditions,  
such as autoimmune disorders, proliferative disorders and infectious diseases.

The invention provides a method for identifying a compound that modulates an HSP-  
 $\alpha$ 2M receptor-mediated process, comprising: (a) contacting a test compound with a heat  
15 shock protein and an alpha (2) macroglobulin receptor; and (b) measuring the level of alpha  
(2) macroglobulin receptor activity or expression, such that if the level of activity or  
expression measured in (b) differs from the level of alpha (2) macroglobulin receptor activity  
in the absence of the test compound, then a compound that modulates an HSP- $\alpha$ 2M  
receptor-mediated process is identified. In one embodiment of this method the compound  
20 identified is an antagonist which interferes with the interaction of the heat shock protein with  
the alpha (2) macroglobulin receptor, further comprising the step of: (c) determining  
whether the level interferes with the interaction of the heat shock protein and the alpha(2)  
macroglobulin receptor. In another embodiment, the test compound is an antibody specific  
for the alpha (2) macroglobulin receptor. In another embodiment, the test compound is an  
25 antibody specific for alpha (2) macroglobulin. In another embodiment, test compound is an  
antibody specific for a heat shock protein. In another embodiment, the test compound is a  
small molecule. In another yet embodiment, the test compound is a peptide. In another  
embodiment, the peptide comprises at least 5 consecutive amino acids of the alpha (2)  
macroglobulin receptor. In yet another embodiment, the peptide comprises at least 5  
30 consecutive amino acids of alpha (2) macroglobulin. In yet another embodiment, the peptide  
comprises at least 5 consecutive amino acids of a heat shock protein sequence. In another  
embodiment, the compound is an agonist which enhances the interaction of the heat shock  
protein with the alpha (2) macroglobulin receptor. In another embodiment, which the HSP-  
 $\alpha$ 2M receptor-mediated process affects an autoimmune disorder, a disease or disorder  
35 involving disruption of antigen presentation or endocytosis, a disease or disorder involving  
cytokine clearance or inflammation, a proliferative disorder, a viral disorder or other  
infectious disease, hypercholesterolemia, Alzheimer's disease, diabetes, or osteoporosis.

The invention also provides a method for identifying a compound that modulates an HSP- $\alpha$ 2M receptor-mediated process, comprising: (a) contacting a test compound with a heat shock protein and an alpha (2) macroglobulin receptor-expressing cell; and (b) measuring the level of alpha (2) macroglobulin receptor activity or expression in the cell, such that if the level of activity or expression measured in (b) differs from the level of alpha (2) macroglobulin receptor activity in the absence of the test compound, then a compound that modulates an HSP- $\alpha$ 2M receptor-mediated process is identified. In yet another embodiment, wherein the alpha (2) macroglobulin receptor activity measured is the ability to interact with a heat shock protein.

The invention also encompasses a method for identifying a compound that modulates the binding of a heat shock protein to the  $\alpha$ 2M receptor, comprising: (a) contacting a heat shock protein with an alpha (2) macroglobulin receptor, or fragment, or analog, derivative or mimetic thereof, in the presence of a test compound; and (b) measuring the amount of heat shock protein bound to the alpha (2) macroglobulin receptor, or fragment, analog, derivative or mimetic thereof, such that if the amount of bound heat shock protein measured in (b) differs from the amount of bound heat shock protein measured in the absence of the test compound, then a compound that modulates the binding of an HSP to the  $\alpha$ 2M receptor is identified. In another embodiment, alpha (2) macroglobulin receptor contacted in step (a) is on a cell surface. In another embodiment, the alpha (2) macroglobulin receptor is immobilized to a solid surface. In another embodiment, the solid surface is a microtiter dish. In another embodiment, the amount of bound heat shock protein is measured by contacting the cell with a heat shock protein-specific antibody. In yet another embodiment, the heat shock protein is labeled and the amount of bound heat shock protein is measured by detecting the label. In another embodiment, the heat shock protein is labeled with a fluorescent label.

The invention further provides a method for identifying a compound that modulates heat shock protein-mediated antigen presentation by alpha (2) macroglobulin receptor-expressing cells comprising: (a) adding a test compound to a mixture of alpha (2) macroglobulin receptor-expressing cells and a complex consisting essentially of a heat shock protein noncovalently associated with an antigenic molecule, under conditions conducive to alpha (2) macroglobulin receptor-mediated endocytosis; (b) measuring the level of antigen-specific stimulation of cytotoxic T cells by alpha (2) macroglobulin receptor-expressing cells, such that if the level measured in (b) differs from the level of said stimulation in the absence of the test compound, then a compound that modulates heat shock protein-mediated antigen presentation by alpha (2) macroglobulin receptor-expressing cells is identified. In one embodiment of this method, the step of measuring the level of the antigenic molecule presented on the cell surface of step (b) comprises: (i) adding the alpha (2) macroglobulin

receptor-expressing cells formed in step (a) to T cells under conditions conducive to the activation of the T cells; and (ii) comparing the level of activation of said cytotoxic T cells with the level of activation of T cells by an alpha (2) macroglobulin receptor-expressing cell formed in the absence of the test compound, wherein an increase or decrease in level of T cell activation indicates that a compound that modulates heat shock protein-mediated antigen presentation by alpha (2) macroglobulin receptor-expressing cells is identified.

In various embodiments, the heat shock protein used in the methods of the invention is gp96. Alternatively, the heat shock proteins hsp90, hsp70, or calreticulin may be used in various embodiments of the invention.

In another embodiment, the invention provides a method for detecting a heat shock protein-alpha (2) macroglobulin receptor-related disorder in a mammal comprising measuring the level of an HSP-alpha (2) macroglobulin receptor-mediated process in a patient sample, such that if the measured level differs from the level found in clinically normal individuals, then a heat shock protein-alpha (2) macroglobulin receptor-related disorder is detected.

The invention also encompasses kits comprising compositions of the invention. In one embodiment, a kit is provided, packaged in one or more containers, comprising: (a) a purified heat shock protein, nucleic acid encoding a heat shock protein, or cell expressing a heat shock protein; and (b) an alpha (2) macroglobulin receptor polypeptide, nucleic acid encoding an alpha (2) macroglobulin receptor polypeptide, or cell expressing an alpha (2) macroglobulin receptor polypeptide. In one embodiment, the kit the alpha (2) macroglobulin receptor polypeptide, nucleic acid encoding an alpha (2) macroglobulin receptor polypeptide, or cell expressing an alpha (2) macroglobulin receptor polypeptide is purified. In another embodiment, the kit further comprises instructions for use in treating an autoimmune disorder, an infectious disease, or a proliferative disorder.

The invention also provides a method for modulating an immune response comprising administering to a mammal a purified compound that modulates the interaction of a heat shock protein with the alpha (2) macroglobulin receptor. In one embodiment, the compound is an agonist which enhances the interaction of the heat shock protein and the alpha (2) macroglobulin receptor. In another embodiment of this method the compound is an antagonist that interferes with the interaction between the heat shock protein and the  $\alpha$ 2M receptor.

The invention further provides a method for treating an autoimmune disorder comprising administering to a mammal in need of such treatment a purified compound that interferes with the interaction of a heat shock protein with the alpha (2) macroglobulin receptor. In one embodiment of this method the compound is an antagonist that interferes with the interaction between the heat shock protein and the  $\alpha$ 2M receptor. In one

embodiment, the antagonist is an antibody specific for alpha (2) macroglobulin receptor. In another embodiment, the antagonist is an antibody specific for a heat shock protein. In another embodiment, the antagonist is a small molecule. In another embodiment, the antagonist is a peptide. In another embodiment, the peptide comprises at least 5 consecutive amino acids of alpha (2) macroglobulin receptor. In another embodiment, the peptide comprises at least 5 consecutive amino acids of alpha (2) macroglobulin. In another embodiment, the peptide comprises at least 5 consecutive amino acids of a heat shock protein sequence.

The invention further provides a method for increasing the immunopotency of a cancer cell or an infected cell comprising transforming said cell with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin receptor polypeptide.

Still further, the invention provides a method for increasing the immunopotency of a cancer cell or an infected cell comprising: (a) transforming said cell with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin receptor polypeptide, and (b) administering said cell to an individual in need of treatment, so as to obtain an elevated immune response.

The invention also provides a recombinant cancer cell transformed with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin receptor polypeptide. In one embodiment, the recombinant cell is a human cell.

In yet another embodiment, the invention provides a recombinant infected cell transformed with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin receptor polypeptide. In one embodiment, the recombinant cell is a human cell.

In another embodiment, the invention provides a method for screening for molecules that specifically bind to an  $\alpha$ 2M receptor comprising the steps of: (a) contacting an  $\alpha$ 2M receptor with one or more test molecules under conditions conducive to binding; and (b) determining whether any of said test molecules specifically bind to the  $\alpha$ 2M receptor. In one embodiment of this method, test molecules are potential immunotherapeutic drugs.

The invention also provides a method for identifying a compound that modulates the binding of an  $\alpha$ 2M receptor ligand to the  $\alpha$ 2M receptor comprising: contacting an  $\alpha$ 2M receptor with an  $\alpha$ 2M receptor ligand, or an  $\alpha$ 2M receptor-binding fragment, analog, derivative, or mimetic thereof, in the presence of one or more test compound; and (b) measuring the amount of  $\alpha$ 2M receptor ligand, or fragment, analog, derivative or mimetic thereof, bound to the  $\alpha$ 2M receptor, such that if the amount of bound  $\alpha$ 2M receptor ligand measured in (b) differs from the amount of bound  $\alpha$ 2M measured in the absence of the test

compound, then a compound that modulates the binding of an  $\alpha 2M$  receptor ligand to the  $\alpha 2M$  receptor is identified.

In another embodiment, a method is provided for identifying a compound that modulates the interaction between the  $\alpha 2M$  receptor and an  $\alpha 2M$  receptor ligand, comprising:  
5 (a) contacting an  $\alpha 2M$  receptor with one or more test compounds; and (b) measuring the level of  $\alpha 2M$  receptor activity or expression, such that if the level of activity or expression measured in (b) differs from the level of  $\alpha 2M$  receptor activity in the absence of one or more test compounds, then a compound that modulates the interaction between the  $\alpha 2M$  receptor and an  $\alpha 2M$  receptor ligand is identified. In one embodiment, the  $\alpha 2M$  receptor ligand is  
10  $\alpha 2M$ .

In another embodiment, a method is provided for identifying a compound that modulates antigen presentation by  $\alpha 2M$  receptor-expressing cells comprising: (a) adding one or more test compounds to a mixture of  $\alpha 2M$  receptor-expressing cells and a complex comprising an  $\alpha 2M$  receptor ligand and an antigenic molecule, under conditions conducive to  
15  $\alpha 2M$  receptor-mediated endocytosis; (b) measuring the level of stimulation of antigen-specific cytotoxic T cells by the  $\alpha 2M$  receptor-expressing cells, such that if the level measured in (b) differs from the level of said stimulation in the absence of the one or more test compounds, then a compound that modulates antigen presentation by  $\alpha 2M$  receptor-expressing cells is identified.

20 In another embodiment, the invention provides a method for modulating an immune response comprising administering to a mammal a purified compound that binds to the  $\alpha 2M$  receptor in an amount effective to modulate an immune response in the mammal.

In yet another embodiment, a method for treating or preventing a disease or disorder is provided comprising administering to a mammal a purified compound that binds to the  
25  $\alpha 2M$  receptor in an amount effective to treat or prevent a disease or disorder in the mammal. In one embodiment, the disease or disorder is cancer or an infectious disease.

In a further embodiment, a method is provided for treating an autoimmune disorder comprising administering to a mammal in need of such treatment a purified compound that binds to the  $\alpha 2M$  receptor in an amount effective to treat an autoimmune disorder in the  
30 mammal.

In another aspect of the invention, a method is provided for stimulating an immune response in a patient comprising administering to said patient blood which has been withdrawn from said patient and treated to remove an  $\alpha 2M$  receptor ligand. In a specific embodiment, the method further comprises administering to said patient a heat shock protein  
35 or a heat shock protein-antigenic peptide complex. In a specific embodiment, blood is administered to said patient by syringe. In another embodiment, said blood is administered to said patient by an intravenous drip.

In another embodiment, a method is provided for stimulating an immune response in a patient comprising: a) removing a  $\alpha$ 2M receptor ligand from blood withdrawn from said patient; and b) returning at least a portion of the  $\alpha$ 2M receptor ligand-depleted blood to said patient.

5 In another embodiment, a method is provided for stimulating an immune response in a patient comprising: a) withdrawing blood from said patient; b) removing a  $\alpha$ 2M receptor ligand from said blood; and c) returning at least a portion of the  $\alpha$ 2M receptor ligand-depleted blood to said patient. In a specific embodiment, the method further comprises after step (a) and before step (c) the step of adding a heat shock protein or a heat shock protein  
10 antigenic-peptide complex to said blood. In a specific embodiment, said blood is returned to said patient by syringe. In another specific embodiment, said blood is returned to said patient by an intravenous drip. In another specific embodiment, the removing a  $\alpha$ 2M receptor ligand from the blood comprises the step of contacting the blood with a solid phase attached to a  $\alpha$ 2M receptor ligand-binding molecule for a time period and under conditions  
15 sufficient to allow binding of  $\alpha$ 2M receptor ligand to the  $\alpha$ 2M receptor ligand-binding molecule solid phase. In another specific embodiment, the  $\alpha$ 2M receptor ligand-binding molecule is  $\alpha$ 2M receptor, or a fragment thereof. In another embodiment, said  $\alpha$ 2M receptor ligand-binding molecule does not bind a heat shock protein. In another embodiment, the  $\alpha$ 2M receptor ligand-binding molecule is an  $\alpha$ 2M receptor ligand-specific antibody, or a  
20 fragment thereof.

In various embodiments, an apheresis system is used in said removing step. In other embodiments blood is withdrawn manually in said withdrawing step. In various embodiments, said removing step comprises separating the plasma from said blood and treating said plasma to remove said  $\alpha$ 2M receptor ligand.

25 The invention further provides a kit comprising in one or more containers a solid phase chromatography column with a purified  $\alpha$ 2M receptor ligand binding molecule attached thereto, such that withdrawn blood can be run over the column to deplete the blood of a  $\alpha$ 2M receptor ligand. In one embodiment, the  $\alpha$ 2M receptor ligand binding molecule of the kit does not bind heat shock proteins.

30 In various embodiments, the  $\alpha$ 2M receptor ligand is  $\alpha$ 2M, a lipoprotein complex, lactoferrin, tissue-type plasminogen activator, urokinase-type plasminogen activator, or an exotoxin.

The term "HSP- $\alpha$ 2M receptor-mediated process" as used herein refers to a process dependent and/or responsive, either directly or indirectly, to the interaction of HSP with the  
35  $\alpha$ 2M receptor. Such processes include processes that result from an aberrant level of expression, synthesis and/or activity of  $\alpha$ 2M receptor, such as endocytic activities relating to the binding of the various  $\alpha$ 2M ligands, including but not limited to HSP,  $\alpha$ 2M, lipoprotein

complexes, lactoferrin, tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), and exotoxins. Such processes include, but are not limited to, endocytosis, antigen presentation, cholesterol regulation, apoE-containing lipoprotein clearance, and chylomicron remnant removal.

5 The terms "HSP- $\alpha$ 2M receptor-related disorder" and "HSP- $\alpha$ 2M receptor-related condition", as used herein, refers to a disorder and a condition, respectively, involving a HSP- $\alpha$ 2M receptor interaction. Such disorders and conditions may result, for example, from an aberrant ability of the  $\alpha$ 2M receptor to interact with HSP, perhaps due to aberrant levels of HSP and/or  $\alpha$ 2M receptor expression, synthesis and/or activity relative to levels found in  
10 normal, unaffected, unimpaired individuals, levels found in clinically normal individuals, and/or levels found in a population whose levels represent a baseline, average HSP and/or  $\alpha$ 2M receptor levels. Such disorders include, but are not limited to, autoimmune disorders, diseases and disorders involving disruption of antigen presentation and/or endocytosis, diseases and disorders involving cytokine clearance and/or inflammation, proliferative  
15 disorders, viral disorders and other infectious diseases, hypercholesterolemia, Alzheimer's disease, diabetes, and osteoporosis.

The term " $\alpha$ 2MR ligand" as used herein, refers to a molecule capable of binding to the  $\alpha$ 2M receptor. Such  $\alpha$ 2MR ligands include as well as known ligands, such as, but not limited to,  $\alpha$ 2M and  $\alpha$ 2M complexes, heat shock proteins and heat shock protein complexes,  
20 lipoprotein complexes, lactoferrin, tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), and exotoxins. In addition,  $\alpha$ 2MR ligands also include molecules which can readily be identified as  $\alpha$ 2MR ligands using standard binding assays well known in the art. Such  $\alpha$ 2MR ligands are typically endocytosed by cell upon binding to the  $\alpha$ 2M receptor.

25

#### 4. BRIEF DESCRIPTION OF THE FIGURES

**FIG. 1A-C .** Identification of an 80 kDa polypeptide as a putative gp96 receptor. **A.** Confocal microscopy of re-presentation-competent RAW264.7 cells stained with gp96-FITC (left panel) and with albumin-FITC (right panel). **B.** SDS-PAGE analysis of detergent  
30 extracts of plasma membranes from surface biotinylated RAW264.7 (re-presentation-competent) or P815 cells (representation-incompetent) eluted from gp96 or albumin-Sepharose (SA) columns and stained with silver stain (top) or avidin-peroxidase (bottom). **C.** gp96-SASD-I<sup>125</sup> was cross-linked to live peritoneal macrophages (MO) or P815 cells, and the cell lysates examined by SDS-PAGE and autoradiography. Various components were  
35 omitted as controls, as indicated.

**FIG. 2A-B.** Anti-p80 antiserum detects an 80 kDa molecule and inhibits re-presentation of gp96-chaperoned AH1 peptide by macrophage. **A.** Pre-immune and immune sera were used to probe blots of plasma membrane extracts of RAW264.7, peritoneal macrophages (both cell types re-presentation-competent), or P815 cells. **B.** Re-presentation of gp96-chaperoned peptide AH1. Sera were added at the final dilution indicated. The solid cross indicates the level of T cell stimulation when the APCs were pulsed directly with the AH1 peptide. The open cross indicates the corresponding value with unpulsed APCs.

**FIG. 3A-C.** Protein microsequencing of the 80 kDa protein. **A.** Analysis of a single tryptic (GALHIYHQR) peptide by tandem- mass spectrometry. All possible b- and y-ion series together with identified b-ion series (red) and y-ion series (blue) are shown. **B.** Collision-induced dissociation (CID) spectrum of this peptide is shown. **C.** Four identified peptides from the  $\alpha 2M$  receptor, peptide mass, and sequence are shown.

**FIG. 4.**  $\alpha 2$ -Macroglobulin inhibits re-presentation of gp96-chaperoned AH1 peptide by macrophage. The solid cross indicates the level of T cell stimulation when the APCs were pulsed directly with the AH1 peptide. The open cross indicates the corresponding value with unpulsed APCs.

**FIG. 5.** Table of specific binding of HSPs and  $\alpha 2$ -macroglobulin to primary cultures and cell lines of several histological origins. The “\*\*\*” indicates percentage of cells staining with FITC over background staining alone. The “#” indicates that the cells were examined by confocal microscopy. All CD11c<sup>+</sup> cells were intensely positive for binding to the three HSPs and  $\alpha 2M$ .

**FIG. 6A-B.** Analysis of cells by flow cytometry for the presence of FITC labelled cells. The macrophage cell lines RAW264.7 (**A**) or RAW309Cr.1 (**B**) were incubated with 100mg/ml of FITC labeled gp96, hsp90, hsp70 or SA. Live cells only were gated based on FSC.

**FIG. 7A-B.** Re-presentation of gp96-chaperoned peptides by APCs that bind HSPs and  $\alpha 2$  macroglobulin. The presence of IFN- $\gamma$  (pg/ml) was assayed as a marker for CTL stimulation. (**A**) Peritoneal macrophage or BM-DCs from C57Bl/6 mice (1X10<sup>4</sup>). (**B**) RAW 264.7 or RAW 309Cr.1 macrophage lines were cultured with gp96 (40 mg/ml) by itself or complexed to the AH1-19 peptide and used to stimulate AH1 specific CTLs (1X10<sup>4</sup>).

**FIG. 8.** Peptides chaperoned by hsp90, CRT, hsp70 and gp96 but not serum albumin are re-presented by RAW264.7 cells. The chaperones, uncomplexed or complexed to the AH1-19 peptide were used to pulse RAW264.7 cells which were tested for their ability to stimulate cognate CTLs.

5

**FIG. 9A-C.** Gp96, hsp90, hsp70 and calreticulin utilize a common receptor for re-presentation. **(A)** RAW264.7 cells were pulsed with gp96-AH1-19 complexes (40 mg/ml gp96) in presence of increasing concentrations of uncomplexed gp96, hsp90, hsp70 or SA. **(B)** Re-presentation of AH1-19 complexed to gp96, hsp90, hsp70, CRT or albumin was carried out in presence of increasing concentrations of  $\alpha$ 2-macroglobulin. The data is plotted as percentage inhibition of re-presentation. **(C)** Re-presentation of AH1-19 complexed to gp96, hsp90, hsp70 or calreticulin in presence of increasing concentrations of anti-CD91 antibody. The data is plotted as percentage inhibition of re-presentation.

10

**FIG. 10A-C.** Re-presentation of gp96-chaperoned peptides follows the classical endogenous antigen presentation pathway. **(A)** Requirement of proteasomes. Peritoneal macrophage (1X106) were either treated or untreated with lactacystin (100 mM). The cells were labeled with chromium and used as targets against VSV8 specific CTLs. **(B)** Requirement of TAP as measured in vitro. Peritoneal macrophage from TAP<sup>+/+</sup> or TAP<sup>-/-</sup> mice were cultured with gp96 or gp96-VSV19 complex and VSV8 specific CTL line. Culture supernatants were tested for the presence of IFN- $\gamma$  (pg/ml) as a marker for CTL stimulation. **(C)** Requirement of TAP as measured in vivo. Gp96-VSV19 complex was injected intraperitoneally. After 10 days, spleens were removed and cells were cultured in vitro with VSV8. The lymphocyte cultures were tested for their ability to lyse EL4 cells (dotted line) or EL4 cells pulsed with VSV8 peptide (solid line). Each line re-presents one mouse.

20

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**FIG. 11.**  $\alpha$ 2M receptor is a sensor of necrotic cell death due to its ability to detect extracellular gp96. Conversely, receptors (psR) for phosphatidyl serine (ps) detect apoptotic cell death.

30

**FIG. 12A.** The mouse  $\alpha$ 2MR cDNA (SEQ ID NO:1) and predicted open reading frame of murine  $\alpha$ 2MR protein (Genbank accession no. CAA47817). **B.** The murine  $\alpha$ 2M protein (SEQ ID NO:2), with residues identified by microsequencing an 80 kDa, gp96-interacting fragment of the receptor highlighted in bold.

35

**FIG. 13A.** The human  $\alpha$ 2M cDNA (SEQ ID NO:3) and predicted open reading frame of  $\alpha$ 2M protein (SEQ ID NO:4)(Genbank accession no. M11313). **B.** The sequence of the

mature human  $\alpha 2M$  protein (SEQ ID NO:5), following cleavage of the N-terminal 23 amino acid signal sequence. Highlighted residues represent the 138 amino acid  $\alpha 2MR$ -binding domain (RBD). Underlined residues represent an extension of the RBD that is present in a  $\alpha 2MR$ -binding, proteolytic fragment of  $\alpha 2M$  (RBDv). Bolded residues have been shown to be important for  $\alpha 2MR$  binding. Italicized residues represent a domain that is conserved among ligands of  $\alpha 2MR$ .

**FIG. 14A.** The human  $\alpha 2MR$  cDNA (SEQ ID NO:6) and predicted open reading frame of human  $\alpha 2MR$  protein (Genbank accession no. NP\_002323). **B.** Primary amino acid sequence of human  $\alpha 2MR$  (SEQ ID NO:7). The approximate locations of complement repeat clusters I and II are highlighted in grey. Individual complement repeats of C1-II are indicated as follows: amino acids of CR3, 5, 7 and 9 are in italics, and amino acids of CR4, 6, 8, and 10 are underlined. Amino acids highlighted in bold were present in an 80kDa peptide fragment of the mouse  $\alpha 2MR$  that bound to gp96. The double underlined residues represent the predicted signal peptide. For the locations of other features of the receptor, such as the EGF repeats, see the article by (Herz *et al.*, 1988, EMBO J. 7:4119-4127).

## 5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to compositions and methods for the use of the alpha (2) macroglobulin receptor (also referred to interchangeably herein as " $\alpha 2MR$ " or "the  $\alpha 2M$  receptor") as a heat shock protein ("HSP") receptor. In particular, the present invention provides compositions comprising isolated  $\alpha 2MR$ - ligand complexes, *e.g.*,  $\alpha 2MR$ -HSP complexes, including isolated and/or recombinant cells, and antibodies, molecules and compounds that modulate the interaction of  $\alpha 2MR$  with an  $\alpha 2MR$  ligand, such as HSP. The invention further encompasses methods for the use of  $\alpha 2MR$  as a heat shock protein receptor, including screening assays to identify compounds that modulate the interaction of  $\alpha 2MR$  with an HSP, or other  $\alpha 2MR$  ligand, and methods for the use of these molecules and complexes for the diagnosis and treatment of immune disorders, proliferative disorders, and infectious diseases.

The term " $\alpha 2MR$  ligand" as used herein, refers to a molecule capable of binding to the  $\alpha 2M$  receptor. Such  $\alpha 2MR$  ligands include as well as known ligands, such as, but not limited to,  $\alpha 2M$  and  $\alpha 2M$  complexes, heat shock proteins and heat shock protein complexes, lipoprotein complexes, lactoferrin, tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), and exotoxins. In addition,  $\alpha 2MR$  ligands also include molecules which can readily be identified as  $\alpha 2MR$  ligands using standard binding assays

well known in the art. Such  $\alpha$ 2MR ligands are typically endocytosed by cell upon binding to  $\alpha$ 2MR.

An HSP useful in the practice of the invention may be selected from among any cellular protein that satisfies any one of the following criteria: the intracellular concentration  
5 of an HSP increases when a cell is exposed to a stressful stimulus; an HSP can bind other proteins or peptides, and can release the bound proteins or peptides in the presence of adenosine triphosphate (ATP) or low pH; or an HSP possesses at least 35% homology with any cellular protein having any of the above properties. Preferably, the HSP used in the compositions and methods of the present invention includes, but are not limited to, HSP90,  
10 gp96, BiP, Hsp70, DnaK, Hsc70, PhoE calreticulin, PDI, or an sHsp, alone or in combination.

In a preferred embodiment, an HSP is a mammalian (*e.g.*, mouse, rat, primate, domestic animal such as dog, cat, cow, horse), and is most preferably, human.

Hsps useful in the practice of the invention include, but are not limited to, members  
15 of the HSP60 family, HSP70 family, HSP90 family, HSP100 family, sHSP family, calreticulin, PDI, and other proteins in the endoplasmic reticulum that contain thioredoxin-like domain(s), such as, but not limited to, ERp72 and ERp61.

HSP analogs, muteins, derivatives, and fragments can also be used in place of HSPs according to the invention. An HSP peptide-binding "fragment" for use in the invention  
20 refers to a polypeptide comprising a HSP peptide-binding domain that is capable of becoming non-covalently associated with a peptide to form a complex that is capable of eliciting an immune response. In one embodiment, an HSP peptide-binding fragment is a polypeptide comprising an HSP peptide-binding domain of approximately 100 to 200 amino acids.

Databases can also be searched to identify sequences with various degrees of  
25 similarities to a query sequence using programs, such as FASTA and BLAST, which rank the similar sequences by alignment scores and statistics. Such nucleotide sequences of non-limiting examples of HSPs that can be used for preparation of the HSPs used in the methods of the invention are as follows: human Hsp70, Genbank Accession No. NM\_005345,  
30 Sargent *et al.*, 1989, Proc. Natl. Acad. Sci. U.S.A., 86:1968-1972; human Hsp90, Genbank Accession No. X15183, Yamazaki *et al.*, Nucl. Acids Res. 17:7108; human gp96: Genbank Accession No. X15187, Maki *et al.*, 1990, Proc. Natl. Acad. Sci., 87: 5658-5562; human BiP: Genbank Accession No. M19645; Ting *et al.*, 1988, DNA 7: 275-286; human Hsp27, Genbank Accession No. M24743; Hickey *et al.*, 1986, Nucleic Acids Res. 14:4127-45;  
35 mouse Hsp70: Genbank Accession No. M35021, Hunt *et al.*, 1990, Gene, 87:199-204; mouse gp96: Genbank Accession No. M16370, Srivastava *et al.*, 1987, Proc. Natl. Acad. Sci., 85:3807-3811; and mouse BiP: Genbank Accession No. U16277, Haas *et al.*, 1988,

Proc. Natl. Acad. Sci. U.S.A., 85: 2250-2254. Due to the degeneracy of the genetic code, the term "HSP sequence", as used herein, refers not only to the naturally occurring amino acid and nucleotide sequence but also encompasses all the other degenerate sequences that encode the HSP.

5           The aforementioned HSP families also contain proteins that are related to HSPs in sequence, for example, having greater than 35% amino acid identity, but whose expression levels are not altered by stress. Therefore, it is contemplated that the definition of heat shock or stress protein, as used herein, embraces other proteins, mutants, analogs, and variants thereof having at least 35% to 55%, preferably 55% to 75%, and most preferably 75% to  
10 85% amino acid identity with members of these families whose expression levels in a cell are enhanced in response to a stressful stimulus. The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. USA  
15 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. USA 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al., 1990, J. Mol. Biol. 215:403-410. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the invention. BLAST protein  
20 searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to a protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, 1997, Nucleic Acids Res.25:3389-3402. Alternatively, PSI-Blast can be used to perform an iterated search which detects distant relationships between molecules (Altschul  
25 *et al.*, 1997, *supra*). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (*e.g.*, XBLAST and NBLAST) can be used (see <http://www.ncbi.nlm.nih.gov>). Another preferred, non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17. Such an algorithm is incorporated into the ALIGN  
30 program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

The immunogenic HSP-peptide complexes of the invention may include any complex containing an HSP and a peptide that is capable of inducing an immune response in a  
35 mammal. The peptides are preferably noncovalently associated with the HSP. Preferred complexes may include, but are not limited to, gp96-peptide complexes, HSP90-peptide complexes, HSP70-peptide complexes, HSP60-peptide complexes, HSP100-peptide

complexes, calreticulin-peptide complexes, and sHSP-peptide complexes. For example, the HSP gp96 which is present in the endoplasmic reticulum of eukaryotic cells and is related to the cytoplasmic HSP90's can be used to generate an effective vaccine containing a gp96-peptide complex.

5 The HSPs,  $\alpha$ 2MR, and/or antigenic molecules for use in the invention can be purified from natural sources, chemically synthesized, or recombinantly produced. Although the HSPs may be allogeneic to the patient, in a preferred embodiment, the HSPs are autologous to the patient to whom they are administered.

## 10 5.1 COMPOSITIONS OF THE INVENTION

The present invention provides compositions that modulate the interaction between  $\alpha$ 2MR and an  $\alpha$ 2MR ligand, such as, for example, an HSP. Such compositions can be used in methods to elicit or modulate an immune response. Such compositions also include antibodies that specifically recognize HSP-  $\alpha$ 2MR complexes, isolated cells that express  
15 HSP- $\alpha$ 2MR complexes, and isolated and recombinant cells that contain recombinant  $\alpha$ 2MR and HSP sequences. In addition, in various methods of the invention, sequences encoding  $\alpha$ 2MR, an HSP, and  $\alpha$ 2M are used for immunotherapy. Such compositions can be used, for example, in immunotherapy against proliferative disorders, infectious diseases, and other HSP- $\alpha$ 2MR-related disorders. Methods for the synthesis and production of such  
20 compositions are described herein.

### 5.1.1 RECOMBINANT EXPRESSION

In various embodiments of the invention, sequences encoding the  $\alpha$ 2MR, an HSP,  $\alpha$ 2M, or other  $\alpha$ 2MR ligand are inserted into an expression vector for propagation and  
25 expression in recombinant cells. Thus, in one embodiment, the  $\alpha$ 2M receptor, HSP,  $\alpha$ 2M, or other  $\alpha$ 2MR ligand coding region is linked to a non-native promoter for expression in recombinant cells.

The amino acid sequence of the portion of  $\alpha$ 2MR that recognizes and binds to HSPs is shown in FIG. 12B (SEQ ID NO:2). Based on the discovery by the Applicant, this  
30 portion of  $\alpha$ 2MR is responsible for recognizing and binding to HSPs and HSP-antigenic peptide complexes. After binding HSPs,  $\alpha$ 2MR facilitates transport of the HSP-antigenic peptide complex into the cell, where the peptide antigens associate with MHC class I molecules and are then presented on the cell surface of the cell, and become available to stimulate an immune response. Based on this invention, compositions comprising agonists  
35 and antagonists of  $\alpha$ 2MR and HSPs interactions can be used to modulate the immune response. Thus, recombinant  $\alpha$ 2MR polypeptides, complexes of  $\alpha$ 2MR and an HSP or HSP-

antigenic peptide complexes, and recombinant cells expressing  $\alpha 2\text{MR}$  or complexes comprising  $\alpha 2\text{MR}$  and antigenic peptides can be used in methods for immunotherapy and diagnostic methods described herein.

In various embodiments of the invention, sequences encoding the  $\alpha 2\text{MR}$ , and/or a heat shock protein or  $\alpha 2\text{M}$ , or fragments thereof, are inserted into an expression vector for propagation and expression in recombinant cells. An expression construct, as used herein, refers to a nucleotide sequence encoding a particular gene product, such as the  $\alpha 2\text{MR}$ , HSP or  $\alpha 2\text{M}$ , operably associated with one or more regulatory regions which allows expression of the encoded gene product in an appropriate host cell. "Operably-associated" refers to an association in which the regulatory regions and the nucleotide sequence encoding the gene product to be expressed are joined and positioned in such a way as to permit transcription, and ultimately, translation.

The DNA may be obtained from known sequences derived from sequence databases by standard procedures known in the art by DNA amplification or molecular cloning directly from a tissue, cell culture, or cloned DNA (e.g., a DNA "library"). Any eukaryotic cell may serve as the nucleic acid source for obtaining the coding region of an hsp gene. Nucleic acid sequences encoding HSPs can be isolated from vertebrate, mammalian, as well as primate sources, including humans. Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. Whatever the source, the hsp gene should be cloned into a suitable vector for propagation of the gene.

Vectors based on *E. coli* are the most popular and versatile systems for high level expression of foreign proteins (Makrides, 1996, Microbiol Rev, 60:512-538). Non-limiting examples of regulatory regions that can be used for expression in *E. coli* may include but not limited to *lac*, *trp*, *lpp*, *phoA*, *recA*, *tac*,  $\lambda P_L$ , and phage T3 and T7 promoters (Makrides, 1996, Microbiol Rev, 60:512-538). Non-limiting examples of prokaryotic expression vectors may include the  $\lambda\text{gt}$  vector series such as  $\lambda\text{gt}11$  (Huynh et al., 1984 in "DNA Cloning Techniques", Vol. I: A Practical Approach (D. Glover, ed.), pp. 49-78, IRL Press, Oxford), and the pET vector series (Studier et al., 1990, Methods Enzymol., 185:60-89). However, a potential drawback of a prokaryotic host-vector system is the inability to perform many of the post-translational processing events of mammalian cells. Thus, an eukaryotic host-vector system is preferred, a mammalian host-vector system is more preferred, and a human host-vector system is the most preferred.

The regulatory regions necessary for transcription of an  $\alpha 2\text{MR}$  sequence, for example, can be provided by the expression vector. A translation initiation codon (ATG) may also be provided to express a nucleotide sequence encoding an  $\alpha 2\text{M}$  receptor that lacks an initiation codon. In a compatible host-construct system, cellular proteins required for

transcription, such as RNA polymerase and transcription factors, will bind to the regulatory regions on the expression construct to effect transcription of the  $\alpha 2\text{MR}$  sequence in the host organism. The precise nature of the regulatory regions needed for gene expression may vary from host cell to host cell. Generally, a promoter is required which is capable of binding  
5 RNA polymerase to initiate the transcription of an operably-associated nucleic acid sequence. Such regulatory regions may include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, the cap site, a CAAT box, and the like. The non-coding region 3' to the coding sequence may contain transcriptional termination regulatory sequences, such as terminators and polyadenylation sites.

10 Both constitutive and inducible regulatory regions may be used for expression of the  $\alpha 2\text{M}$  receptor, HSP,  $\alpha 2\text{M}$ , or other  $\alpha 2\text{MR}$  ligand. It may be desirable to use inducible promoters when the conditions optimal for growth of the recombinant cells and the conditions for high level expression of the gene product are different. Examples of useful regulatory regions are provided in the next section below.

15 For expression of the  $\alpha 2\text{M}$  receptor, HSP,  $\alpha 2\text{M}$ , or other  $\alpha 2\text{MR}$  ligand gene product in mammalian host cells, a variety of regulatory regions can be used, for example, the SV40 early and late promoters, the cytomegalovirus (CMV) immediate early promoter, and the Rous sarcoma virus long terminal repeat (RSV-LTR) promoter. Inducible promoters that may be useful in mammalian cells include but are not limited to those associated with the  
20 metallothionein II gene, mouse mammary tumor virus glucocorticoid responsive long terminal repeats (MMTV-LTR), the  $\beta$ -interferon gene, and the Hsp70 gene (Williams *et al.*, 1989, *Cancer Res.* 49:2735-42; Taylor *et al.*, 1990, *Mol. Cell Biol.*, 10:165-75). It may be advantageous to use heat shock promoters or stress promoters to drive expression of  $\alpha 2\text{MR}$  in recombinant host cells.

25 The following animal regulatory regions, which exhibit tissue specificity and have been utilized in transgenic animals, can also be used in tumor cells of a particular tissue type: elastase I gene control region which is active in pancreatic acinar cells (Swift *et al.*, 1984, *Cell* 38:639-646; Ornitz *et al.*, 1986, *Cold Spring Harbor Symp. Quant. Biol.* 50:399-409; MacDonald, 1987, *Hepatology* 7:425-515); insulin gene control region which is active in  
30 pancreatic beta cells (Hanahan, 1985, *Nature* 315:115-122), immunoglobulin gene control region which is active in lymphoid cells (Grosschedl *et al.*, 1984, *Cell* 38:647-658; Adames *et al.*, 1985, *Nature* 318:533-538; Alexander *et al.*, 1987, *Mol. Cell. Biol.* 7:1436-1444), mouse mammary tumor virus control region which is active in testicular, breast, lymphoid and mast cells (Leder *et al.*, 1986, *Cell* 45:485-495), albumin gene control region which is  
35 active in liver (Pinkert *et al.*, 1987, *Genes and Devel.* 1:268-276), alpha-fetoprotein gene control region which is active in liver (Krumlauf *et al.*, 1985, *Mol. Cell. Biol.* 5:1639-1648; Hammer *et al.*, 1987, *Science* 235:53-58; alpha 1-antitrypsin gene control region which is

active in the liver (Kelsey et al., 1987, *Genes and Devel.* 1:161-171), beta-globin gene control region which is active in myeloid cells (Mogram et al., 1985, *Nature* 315:338-340; Kollias et al., 1986, *Cell* 46:89-94; myelin basic protein gene control region which is active in oligodendrocyte cells in the brain (Readhead et al., 1987, *Cell* 48:703-712); myosin light chain-2 gene control region which is active in skeletal muscle (Sani, 1985, *Nature* 314:283-286), and gonadotropic releasing hormone gene control region which is active in the hypothalamus (Mason et al., 1986, *Science* 234:1372-1378).

The efficiency of expression of the  $\alpha 2M$  receptor in a host cell may be enhanced by the inclusion of appropriate transcription enhancer elements in the expression vector, such as those found in SV40 virus, Hepatitis B virus, cytomegalovirus, immunoglobulin genes, metallothionein,  $\beta$ -actin (see Bittner et al., 1987, *Methods in Enzymol.* 153:516-544; Gorman, 1990, *Curr. Op. in Biotechnol.* 1:36-47).

The expression vector may also contain sequences that permit maintenance and replication of the vector in more than one type of host cell, or integration of the vector into the host chromosome. Such sequences may include but are not limited to replication origins, autonomously replicating sequences (ARS), centromere DNA, and telomere DNA. It may also be advantageous to use shuttle vectors that can be replicated and maintained in at least two types of host cells.

In addition, the expression vector may contain selectable or screenable marker genes for initially isolating or identifying host cells that contain DNA encoding an  $\alpha 2M$  receptor. For long term, high yield production of  $\alpha 2M$  receptor, stable expression in mammalian cells is preferred. A number of selection systems may be used for mammalian cells, including, but not limited, to the Herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalski and Szybalski, 1962, *Proc. Natl. Acad. Sci. USA* 48:2026), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes can be employed in *tk*, *hgprt* or *aprt* cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for dihydrofolate reductase (*dhfr*), which confers resistance to methotrexate (Wigler et al., 1980, *Natl. Acad. Sci. USA* 77:3567; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neomycin phosphotransferase (*neo*), which confers resistance to the aminoglycoside G-418 (Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1); and hygromycin phosphotransferase (*hyg*), which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Other selectable markers, such as but not limited to histidinol and Zeocin<sup>TM</sup> can also be used.

In order to insert the DNA sequence encoding  $\alpha 2M$  receptor, HSP,  $\alpha 2M$ , or other  $\alpha 2MR$  ligand into the cloning site of a vector, DNA sequences with regulatory functions, such as promoters, must be attached to DNA sequences encoding the  $\alpha 2M$  receptor, HSP,

$\alpha$ 2M, or other  $\alpha$ 2MR ligand, respectively. To do this, linkers or adapters providing the appropriate compatible restriction sites may be ligated to the ends of cDNA or synthetic DNA encoding an  $\alpha$ 2M receptor, by techniques well known in the art (Wu et al., 1987, Methods in Enzymol 152:343-349). Cleavage with a restriction enzyme can be followed by  
5 modification to create blunt ends by digesting back or filling in single-stranded DNA termini before ligation. Alternatively, a desired restriction enzyme site can be introduced into a fragment of DNA by amplification of the DNA by use of PCR with primers containing the desired restriction enzyme site.

In one embodiment, an expression construct comprising an  $\alpha$ 2M receptor sequence  
10 operably associated with regulatory regions can be directly introduced into appropriate host cells for expression and production of  $\alpha$ 2MR without further cloning (see, for example, U.S. Patent No. 5,580,859). The expression constructs may also contain DNA sequences that facilitate integration of the  $\alpha$ 2M receptor sequence into the genome of the host cell, e.g., via homologous recombination. In this instance, it is not necessary to employ an expression  
15 vector comprising a replication origin suitable for appropriate host cells in order to propagate and express the  $\alpha$ 2M receptor in the host cells.

Expression constructs containing cloned nucleotide sequence encoding the  $\alpha$ 2M receptor, an HSP,  $\alpha$ 2M, or other  $\alpha$ 2MR ligand, can be introduced into the host cell by a variety of techniques known in the art, including but not limited to, for prokaryotic cells,  
20 bacterial transformation (Hanahan, 1985, in DNA Cloning, A Practical Approach, 1:109-136), and for eukaryotic cells, calcium phosphate mediated transfection (Wigler et al., 1977, Cell 11:223-232), liposome-mediated transfection (Schaefer-Ridder et al., 1982, Science 215:166-168), electroporation (Wolff et al., 1987, Proc Natl Acad Sci 84:3344), and microinjection (Cappechi, 1980, Cell 22:479-488).

For long term, high yield production of properly processed  $\alpha$ 2M receptor, HSP,  $\alpha$ 2M,  
25 or other  $\alpha$ 2MR ligand, stable expression in mammalian cells is preferred. Cell lines that stably express the  $\alpha$ 2M receptor, HSP,  $\alpha$ 2M, or other  $\alpha$ 2MR ligand or  $\alpha$ 2MR-peptide complexes may be engineered by using a vector that contains a selectable marker. By way of example but not limitation, following the introduction of the expression constructs,  
30 engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the expression construct confers resistance to the selection and optimally allows cells to stably integrate the expression construct into their chromosomes and to grow in culture and to be expanded into cell lines. Such cells can be cultured for a long period of time while the desired gene product is  
35 expressed continuously.

The recombinant cells may be cultured under standard conditions of temperature, incubation time, optical density, and media composition. Alternatively, recombinant

antigenic cells may be cultured under conditions emulating the nutritional and physiological requirements of the cancer cell or infected cell. However, conditions for growth of recombinant cells may be different from those for expression of the  $\alpha 2M$  receptor, HSPs,  $\alpha 2M$ , or other  $\alpha 2MR$  ligand, or antigenic peptide.

5

### 5.1.2 PEPTIDE SYNTHESIS

An alternative to producing peptides and polypeptides comprising HSP,  $\alpha 2M$  receptor,  $\alpha 2M$  or other  $\alpha 2MR$  ligand sequences, by recombinant techniques is peptide synthesis. For example, a peptide corresponding to a portion of an HSP or an  $\alpha 2M$  peptide  
10 comprising the receptor-binding domain, which can be used as an antagonist in the therapeutic methods described herein, can be synthesized by use of a peptide synthesizer. Synthetic peptides corresponding to  $\alpha 2M$  receptor sequences useful for therapeutic methods described herein can also be produced synthetically. Conventional peptide synthesis may be used or other synthetic protocols well known in the art.

15 For example, peptides having the amino acid sequence of the  $\alpha 2M$  receptor, an HSP,  $\alpha 2M$ , or other  $\alpha 2MR$  ligand, or an analog, mutein, fragment, or derivative thereof, may be synthesized by solid-phase peptide synthesis using procedures similar to those described by Merrifield, 1963, J. Am. Chem. Soc., 85:2149. During synthesis, N- $\alpha$ -protected amino acids having protected side chains are added stepwise to a growing polypeptide chain linked by its  
20 C-terminal and to an insoluble polymeric support i.e., polystyrene beads. The peptides are synthesized by linking an amino group of an N- $\alpha$ -deprotected amino acid to an  $\alpha$ -carboxyl group of an N- $\alpha$ -protected amino acid that has been activated by reacting it with a reagent such as dicyclohexylcarbodiimide. The attachment of a free amino group to the activated carboxyl leads to peptide bond formation. The most commonly used N- $\alpha$ -protecting groups  
25 include Boc which is acid labile and Fmoc which is base labile. Details of appropriate chemistries, resins, protecting groups, protected amino acids and reagents are well known in the art and so are not discussed in detail herein (*See, Atherton, et al., 1989, Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, and Bodanszky, 1993, Peptide Chemistry, A Practical Textbook, 2nd Ed., Springer-Verlag*).

30 Purification of the resulting  $\alpha 2M$  receptor, HSP,  $\alpha 2M$ , or other  $\alpha 2MR$  ligand peptides is accomplished using conventional procedures, such as preparative HPLC using gel permeation, partition and/or ion exchange chromatography. The choice of appropriate matrices and buffers are well known in the art and so are not described in detail herein.

In addition, analogs and derivatives of  $\alpha 2M$  receptor, HSP,  $\alpha 2M$ , or other  $\alpha 2MR$   
35 ligand protein can be chemically synthesized. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the

$\alpha$ 2M receptor, HSP,  $\alpha$ 2M, or other  $\alpha$ 2MR ligand sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu,  $\epsilon$ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline,  
5 hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids such as  $\beta$ -methyl amino acids, C $\alpha$ -methyl amino acids, N $\alpha$ -methyl amino acids, and amino acid analogs in general.

### 10 5.1.3 ANTIBODIES SPECIFIC FOR $\alpha$ 2M RECEPTOR-HSP COMPLEXES

Described herein are methods for the production of antibodies capable of specifically recognizing  $\alpha$ 2M receptor epitopes, HSP- $\alpha$ 2M receptor complex epitopes or epitopes of conserved variants or peptide fragments of the receptor or receptor complexes. Such antibodies are useful for therapeutic and diagnostic methods of the invention.

15 Such antibodies may include, but are not limited to, polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')<sub>2</sub> fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Such antibodies may be used, for example, in the detection of an  $\alpha$ 2M receptor or HSP- $\alpha$ 2M  
20 receptor complex in an biological sample. Such antibodies may also be utilized in conjunction with, for example, compound screening schemes, as described below, in Section 5.2, for the evaluation of the effect of test compounds on the interaction between HSPs and the  $\alpha$ 2M receptor.

Anti- $\alpha$ 2M receptor complex antibodies may additionally be used as a method for the  
25 inhibition of abnormal receptor product activity. Thus, such antibodies may, be utilized as part of treatment methods for HSP- $\alpha$ 2M receptor related disorders, *e.g.*, autoimmune disorders.

For the production of antibodies against  $\alpha$ 2M receptor or receptor complexes, various host animals may be immunized by injection with an  $\alpha$ 2M receptor or HSP- $\alpha$ 2M receptor  
30 complex, or a portion thereof. An antigenic portion of  $\alpha$ 2M receptor or HSP- $\alpha$ 2M receptor complex can be readily predicted by algorithms known in the art.

Host animals may include, but are not limited to rabbits, mice, and rats, to name but a few. Various adjuvants may be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), mineral  
35 gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, and

potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*.

5 Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of animals immunized with an antigen, such as an  $\alpha 2M$  receptor or HSP- $\alpha 2M$  receptor complex, or an antigenic functional derivative thereof. For the production of polyclonal antibodies, host animals such as those described above, may be immunized by injection with  $\alpha 2M$  receptor or HSP- $\alpha 2M$  receptor complex, or portion thereof, supplemented with adjuvants as also described above.

10 Monoclonal antibodies, which are homogeneous populations of antibodies to a particular antigen, may be obtained by any technique that provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique of Kohler and Milstein, (1975, Nature 256, 495-497; and U.S. Patent No. 4,376,110), the human B-cell hybridoma technique (Kosbor *et al.*, 1983, Immunology Today 4: 72; Cole *et al.*, 1983, Proc. Natl. Acad. Sci. USA 80, 2026-2030), and the EBV-hybridoma technique (Cole *et al.*, 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAb of this invention may be cultivated *in vitro* or *in vivo*. Production of high titers of mAbs *in vivo* makes this the presently preferred method of production.

20 In addition, techniques developed for the production of "chimeric antibodies" (Morrison, *et al.*, 1984, Proc. Natl. Acad. Sci., 81: 6851-6855; Neuberger, *et al.*, 1984, Nature 312: 604-608; Takeda, *et al.*, 1985, Nature, 314: 452-454) by splicing the genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region (see, *e.g.*, Cabilly *et al.*, U.S. Patent No. 4,816,567; and Boss *et al.*, U.S. Patent No. 4,816,397, which are incorporated herein by reference in their entirety).

30 In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals (see PCT International Publication No. WO 89/12690, published December 12, 1989). According to the invention, human antibodies may be used and can be obtained by using human hybridomas (Cote *et al.*, 1983, Proc. Natl. Acad. Sci. U.S.A. 80:2026-2030) or by transforming human B cells with EBV virus *in vitro* (Cole *et al.*, 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, pp. 77-96). Techniques developed for the production of "chimeric antibodies" (Morrison *et al.*, 1984, Proc. Natl. Acad. Sci. U.S.A. 81:6851-6855; Neuberger *et al.*, 1984, Nature 312:604-608; Takeda *et al.*,

1985, Nature 314:452-454) by splicing the genes from a mouse antibody molecule specific for an  $\alpha 2M$  receptor-HSP complex together with genes from a human antibody molecule of appropriate biological activity can also be used; such antibodies are within the scope of this invention.

5 Humanized antibodies are also provided (see U.S. Patent No. 5,225,539 by Winter). An immunoglobulin light or heavy chain variable region consists of a "framework" region interrupted by three hypervariable regions, referred to as complementarity determining regions (CDRs). The extent of the framework region and CDRs have been precisely defined (see, "Sequences of Proteins of Immunological Interest", Kabat, E. *et al.*, U.S. Department of  
10 Health and Human Services (1983). Briefly, humanized antibodies are antibody molecules from non-human species having one or more CDRs from the non-human species and a framework region from a human immunoglobulin molecule. Such CDRS-grafted antibodies have been successfully constructed against various antigens, for example, antibodies against IL-2 receptor as described in Queen *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86:10029;  
15 antibodies against the cell surface receptor CAMPATH as described in Riechmann *et al.*, 1988, Nature 332:323; antibodies against hepatitis B in Co *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:2869; as well as against viral antigens of the respiratory syncytial virus in Tempest *et al.*, 1991, Bio-Technology 9:267. Humanized antibodies are most preferred for therapeutic use in humans.

20 Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778; Bird, 1988, Science 242: 423-426; Huston *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85: 5879-5883; and Ward *et al.*, 1989, Nature 334: 544-546) can be adapted to produce single chain antibodies against  $\alpha 2M$  receptor or HSP- $\alpha 2M$  receptor complexes, or portions thereof. Single chain antibodies are formed by linking the heavy and light chain  
25 fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide.

Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')<sub>2</sub> fragments, which can be produced by pepsin digestion of the antibody molecule and the Fab fragments, which can be generated by reducing the disulfide bridges of the F(ab')<sub>2</sub> fragments.  
30 Alternatively, Fab expression libraries may be constructed (Huse *et al.*, 1989, Science, 246: 1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

Antibodies to the  $\alpha 2M$  receptor can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" the  $\alpha 2M$  receptor, using techniques well known to those skilled in  
35 the art (see, *e.g.*, Greenspan & Bona, 1993, FASEB J 7(5):437-444; and Nissinoff, 1991, J. Immunol. 147(8):2429-2438). For example antibodies which bind to the  $\alpha 2M$  receptor ECD and competitively inhibit the binding of HSPs to the  $\alpha 2M$  receptor can be used to generate

anti-idiotypes that "mimic" the ECD and, therefore, bind and neutralize HSPs. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize the native ligand and treat HSP- $\alpha$ 2M receptor-related disorders, such as immunological disorders, proliferative disorders, and infectious diseases.

5           Alternatively, antibodies to the  $\alpha$ 2M receptor that can act as agonists of the  $\alpha$ 2M receptor activity can be generated. Such antibodies will bind to the  $\alpha$ 2M receptor and activate the signal transducing activity of the receptor. In addition, antibodies that act as antagonist of the  $\alpha$ 2M receptor activity, *i.e.* inhibit the activation of the  $\alpha$ 2M receptor would be particularly useful for treating autoimmune disorders, proliferative disorders, such as  
10 cancer, and infectious diseases. Methods for assaying for such agonists and antagonists are described in detail in Section 5.2, below.

## 5.2    ASSAYS FOR THE IDENTIFICATION OF COMPOUNDS THAT INTERACT WITH THE $\alpha$ 2M RECEPTOR

15           The present invention is based on the discovery that the  $\alpha$ 2M receptor recognizes HSP-antigenic peptide complexes and transports them within the cell for the purpose of presenting such antigenic molecules to cells of the immune system and eliciting an immune response. Thus, methods for identifying compounds that interact with the receptor, or  
20 enhance or block the function of the receptor, are included in the invention. The present invention provides *in vitro* and *in vivo* assay systems, described in the subsections below, which can be used to identify compounds or compositions that interact with the  $\alpha$ 2M receptor, or modulate the activity of the  $\alpha$ 2M receptor and its interaction with HSPs or HSP-peptide complexes.

25           The invention provides screening methodologies useful in the identification of small molecules, proteins and other compounds which interact with the  $\alpha$ 2M receptor, or modulate the interaction of HSPs with the  $\alpha$ 2M receptor. Such compounds may bind the  $\alpha$ 2M receptor genes or gene products with differing affinities, and may serve as regulators of receptor activity *in vivo* with useful therapeutic applications in modulating the immune  
30 response. For example, certain compounds that inhibit receptor function may be used in patients to downregulate destructive immune responses which are caused by cellular release of HSPs.

          Methods to screen potential agents for their ability to interact with the  $\alpha$ 2M receptor, or modulate  $\alpha$ 2M receptor expression and activity can be designed based on the inventor's  
35 discovery of the receptor and its role in HSP or HSP-peptide complex binding and recognition.  $\alpha$ 2M receptor protein, nucleic acids, and derivatives can be used in screening assays to detect molecules that specifically bind to HSP proteins, derivatives, or nucleic

acids, and thus have potential use as agonists or antagonists of the  $\alpha 2M$  receptor, to modulate the immune response. In a preferred embodiment, such assays are performed to screen for molecules with potential utility as anti-autoimmune disease, anti-cancer and anti-infective drugs (such as anti-viral drugs and antibiotic drugs), or lead compounds for drug  
5 development. For example, recombinant cells expressing  $\alpha 2M$  receptor nucleic acids can be used to recombinantly produce  $\alpha 2M$  receptor in these assays, to screen for molecules that interfere with the binding of HSPs to the  $\alpha 2M$  receptor. Similar methods can be used to screen for molecules that bind to the  $\alpha 2M$  receptor derivatives or nucleic acids. Methods that can be used to carry out the foregoing are commonly known in the art.

10 Compounds capable of specifically binding the  $\alpha 2M$  receptor can be useful for immunotherapy. In one embodiment, an assay is disclosed for identifying compounds that specifically bind the  $\alpha 2M$  receptor comprising: (a) contacting an  $\alpha 2M$  receptor with one or more test compounds under conditions conducive to binding; and (b) identifying one or more test compounds which specifically bind to the  $\alpha 2M$  receptor, such that a compound capable  
15 of specifically binding the  $\alpha 2M$  receptor is identified as a compound useful for immunotherapy.

Another method encompassed by the invention for identifying a compound useful for immunotherapy involves identifying a compound which modulates the binding of an  $\alpha 2M$  receptor ligand to the  $\alpha 2M$  receptor. The term " $\alpha 2M$  receptor ligand" as used herein, refers  
20 to an molecule capable of binding to the  $\alpha 2M$  receptor. Such  $\alpha 2M$  receptor ligands include, but are not limited to,  $\alpha 2M$  and  $\alpha 2M$  complexes, heat shock proteins and heat shock protein complexes, lipoprotein complexes, lactoferrin, tissue-type plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), and exotoxins. Such ligands are typically endocytosed by cell upon binding to the  $\alpha 2M$  receptor. The method comprises the steps of:  
25 (a) contacting an  $\alpha 2M$  receptor with an  $\alpha 2M$  receptor ligand, or fragment, or analog, derivative or mimetic thereof, in the presence of one or more test compound; and (b) measuring the amount of  $\alpha 2M$  receptor ligand, or fragment, analog, derivative or mimetic thereof, bound to the  $\alpha 2M$  receptor, such that if the amount of bound  $\alpha 2M$  receptor ligand  
30 measured in (b) differs from the amount of bound  $\alpha 2M$  receptor measured in the absence of the test compound, then a compound useful for immunotherapy that modulates the binding of an  $\alpha 2M$  receptor ligand to the  $\alpha 2M$  receptor is identified.

In another embodiment, a method for identifying a compound useful for immunotherapy which modulates the interaction between the  $\alpha 2M$  receptor and an  $\alpha 2M$  receptor ligand is provided by the invention. This method comprises the steps of: (a)  
35 contacting an  $\alpha 2M$  receptor with one or more test compounds; and (b) measuring the level of  $\alpha 2M$  receptor activity or expression, such that if the level of activity or expression measured in (b) differs from the level of  $\alpha 2M$  receptor activity in the absence of one or more test

compounds, then a compound that modulates the interaction between the  $\alpha 2M$  receptor and an  $\alpha 2M$  receptor ligand is identified.

In another embodiment, an assay for identifying a compound that modulates an HSP- $\alpha 2M$  receptor-mediated process is disclosed. This assay comprises: (a) contacting a test  
5 compound with an HSP and an  $\alpha 2M$  receptor; and (b) measuring the level of  $\alpha 2M$  receptor activity or expression, such that if the level of activity or expression measured in (b) differs from the level of  $\alpha 2M$  receptor activity in the absence of the test compound, then a  
compound that modulates an HSP- $\alpha 2M$  receptor-mediated process is identified. In another  
embodiment, in which the compound identified is an antagonist which interferes with the  
10 interaction of the HSP with the  $\alpha 2M$  receptor, the method further comprises the step of determining whether the level interferes with the interaction of the HSP and the  $\alpha 2M$  receptor.

In another embodiment, a cell-based method for identifying a compound that modulates an HSP- $\alpha 2M$  receptor-mediated process is described. This method comprises the  
15 following steps: (a) contacting a test compound with a heat shock protein and an  $\alpha 2M$  receptor-expressing cell; and (b) measuring the level of  $\alpha 2M$  receptor activity or expression in the cell, such that if the level of activity or expression measured in (b) differs from the level of  $\alpha 2M$  receptor activity in the absence of the test compound, then a compound that modulates an HSP- $\alpha 2M$  receptor-mediated process is identified.

In another embodiment, a receptor-ligand binding assay for identifying a compound that interacts with  $\alpha 2MR$ , or modulates the binding of an HSP to  $\alpha 2MR$ . One such method  
20 comprises: (a) contacting an HSP with an  $\alpha 2M$  receptor, or fragment, or analog, derivative or mimetic thereof, in the presence of a test compound; and (b) measuring the amount of heat shock protein bound to the  $\alpha 2M$  receptor, or fragment, analog, derivative or mimetic thereof,  
25 such that if the amount of bound heat shock protein measured in (b) differs from the amount of bound heat shock protein measured in the absence of the test compound, then a compound that modulates the binding of an HSP to the  $\alpha 2M$  receptor is identified.

In another embodiment, a method for identifying a compound that modulates antigen presentation by  $\alpha 2MR$ -expressing cells is provided by the invention. In one embodiment,  
30 such a method comprises: (a) adding one or more test compounds to a mixture of  $\alpha 2MR$ -expressing cells and a complex comprising an  $\alpha 2MR$  ligand and an antigenic molecule, under conditions conducive to  $\alpha 2MR$ -mediated endocytosis; (2) measuring the level of stimulation of antigen-specific cytotoxic T cells by the  $\alpha 2MR$ -expressing cells, such that if the level measured in (b) differs from the level of said stimulation in the absence of the one  
35 or more test compounds, then a compound that modulates antigen presentation by  $\alpha 2MR$ -expressing cells is identified. In another embodiment, a test compound is added to a mixture of  $\alpha 2MR$ -expressing cells and a complex consisting essentially of an HSP noncovalently

associated with an antigenic molecule, under conditions conducive to  $\alpha$ 2MR-mediated endocytosis; and the level of stimulation of antigen-specific cytotoxic T cells by the  $\alpha$ 2MR-expressing cells is measured, such that if the level measured differs from the level of said stimulation in the absence of the test compound, then a compound that modulates HSP-mediated antigen presentation by  $\alpha$ 2MR-expressing cells is identified.

The assays of the present invention may be first optimized on a small scale (*i.e.*, in test tubes), and then scaled up for high-throughput assays. In various embodiments, the *in vitro* screening assays of the present invention may be performed using purified components or cell lysates. In other embodiments, the screening assays may be carried out in intact cells in culture and in animal models. In accordance with the present invention, test compounds which are shown to modulate the activity of the  $\alpha$ 2M receptor as described herein *in vitro*, will further be assayed *in vivo*, including cultured cells and animal models to determine if the test compound has the similar effects *in vivo* and to determine the effects of the test compound on antigen presentation, cytokine release, intracellular  $\text{Ca}^{++}$  release, T-cell cytotoxicity, tumor progression, the accumulation or degradation of positive and negative regulators, cellular proliferation, *etc.*

#### 5.2.1 $\alpha$ 2M RECEPTOR-LIGAND BINDING ASSAYS

The screening assays, described herein, can be used to identify compounds and compositions, including peptides and organic, non-protein molecules that interact with the  $\alpha$ 2M receptor, or that modulate the interaction between HSPs and the  $\alpha$ 2M receptor. Recombinant, synthetic, and otherwise exogenous compounds may have binding capacity and, therefore, may be candidates for pharmaceutical agents. Alternatively, the proteins and compounds include endogenous cellular components which interact with the identified genes and proteins *in vivo*. Such endogenous components may provide new targets for pharmaceutical and therapeutic interventions.

Thus, in a preferred embodiment, both naturally occurring and/or synthetic compounds (*e.g.*, libraries of small molecules or peptides), may be screened for interacting with  $\alpha$ 2M receptor and/or modulating  $\alpha$ 2M receptor activity. In another series of embodiments, cell lysates or tissue homogenates may be screened for proteins or other compounds which bind to one of the normal or mutant  $\alpha$ 2M receptor genes and  $\alpha$ 2M receptor polypeptides.

The screening assays described herein may be used to identify small molecules, peptides or proteins, or derivatives, analogs and fragments thereof, that interact with and/or modulate the interaction of HSPs with the  $\alpha$ 2M receptor. Such compounds may be used as agonists or antagonists of the uptake of  $\alpha$ 2M receptor ligands, such as HSPs and HSP

complexes, by the cell surface receptor. For example, compounds that modulate the  $\alpha$ 2M receptor-ligand interaction include, but are not limited to, compounds that bind to the  $\alpha$ 2M receptor, thereby either inhibiting (antagonists) or enhancing (agonists) the binding of ligands, such as HSPs and HSP complexes, to the receptor, as well as compounds that bind to the ligand, such as for example, HSPs, thereby preventing or enhancing binding of ligand to the receptor. Compounds that affect  $\alpha$ 2M receptor gene activity (by affecting  $\alpha$ 2M receptor gene expression, including molecules, *e.g.*, proteins or small organic molecules, that affect transcription or interfere with splicing events so that expression of the full length or truncated forms of  $\alpha$ 2M receptor can be modulated) can also be identified in the screens of the invention. Further, it should be noted that the assays described can also identify compounds that modulate  $\alpha$ 2M receptor ligand, for example HSP, uptake by  $\alpha$ 2M receptor (*e.g.*, compounds which affect downstream signaling in the  $\alpha$ 2M receptor signal transduction pathway). The identification and use of such compounds which affect signaling events downstream of the  $\alpha$ 2M receptor and thus modulate effects of the receptor on the immune response are within the scope of the invention.

Compounds that affect the  $\alpha$ 2M receptor gene activity (by affecting the  $\alpha$ 2M receptor gene expression, including molecules, *e.g.*, proteins or small organic molecules, that affect transcription or interfere with splicing events so that expression of the full length or the truncated form of the  $\alpha$ 2M receptor can be modulated) can also be identified in the screens of the invention. However, it should be noted that the assays described can also identify compounds that modulate the  $\alpha$ 2M receptor signal transduction (*e.g.*, compounds which affect downstream signaling events, such as inhibitors or enhancers of endocytic activity which is activated by ligand binding to the  $\alpha$ 2M receptor). The identification and use of such compounds which affect signaling events downstream of the  $\alpha$ 2M receptor and thus modulate effects of the  $\alpha$ 2M receptor on the allergenic response are within the scope of the invention.

The screening assays described herein are designed to detect compounds that modulate, *i.e.* interfere with or enhance, ligand-receptor interactions, including HSP- $\alpha$ 2M receptor interactions. As described in detail below, such assays are functional assays, such as binding assays, that can be adapted to a high-throughput screening methodologies.

Binding assays can be used to identify compounds that modulate the interaction between ligands, for example, HSPs, and the  $\alpha$ 2M receptor. In one aspect of the invention the screens may be designed to identify compounds that disrupt the interaction between the  $\alpha$ 2M receptor and a ligand, such as, for example, HSPs or peptides derived from an HSP,  $\alpha$ 2M, or another  $\alpha$ 2M receptor ligand. Such compounds will be useful as lead compounds for antagonists of HSP- $\alpha$ 2M receptor-related disorders and conditions, such as immune disorders, proliferative disorders, and infectious diseases.

Binding assays may be performed either as direct binding assays or as competition binding assays. In a direct binding assay, a test compound is tested for binding either to the  $\alpha$ 2M receptor or to an  $\alpha$ 2M receptor ligand, such as an HSP. Then, in a second step, the test compound is tested for its ability to modulate the ligand- $\alpha$ 2M receptor interaction.

- 5 Competition binding assays, on the other hand, assess the ability of a test compound to compete with a ligand, *i.e.* an HSP, for binding to the  $\alpha$ 2M receptor.

In a direct binding assay, either the ligand and/or the  $\alpha$ 2M receptor is contacted with a test compound under conditions that allow binding of the test compound to the ligand or the receptor. The binding may take place in solution or on a solid surface. Preferably, the test  
10 compound is previously labeled for detection. Any detectable compound may be used for labeling, such as but not limited to, a luminescent, fluorescent, or radioactive isotope or group containing same, or a nonisotopic label, such as an enzyme or dye. After a period of incubation sufficient for binding to take place, the reaction is exposed to conditions and manipulations that remove excess or non-specifically bound test compound. Typically, it  
15 involves washing with an appropriate buffer. Finally, the presence of a ligand-test compound (*e.g.*, HSP-test compound) or a the  $\alpha$ 2M receptor-test compound complex is detected.

In a competition binding assay, test compounds are assayed for their ability to disrupt or enhance the binding of the ligand (*e.g.*, HSP) to the  $\alpha$ 2M receptor. Labeled ligand (*e.g.*,  
20 HSP) may be mixed with the  $\alpha$ 2M receptor or fragment or derivative thereof, and placed under conditions in which the interaction between them would normally occur, with and without the addition of the test compound. The amount of labeled ligand (*e.g.*, HSP) that binds the  $\alpha$ 2M receptor may be compared to the amount bound in the presence or absence of test compound.

25 In a preferred embodiment, to facilitate complex formation and detection, the binding assay is carried out with one or more components immobilized on a solid surface. In various embodiments, the solid support could be, but is not restricted to, polycarbonate, polystyrene, polypropylene, polyethylene, glass, nitrocellulose, dextran, nylon, polyacrylamide and agarose. The support configuration can include beads, membranes,  
30 microparticles, the interior surface of a reaction vessel such as a microtiter plate, test tube or other reaction vessel. The immobilization of the  $\alpha$ 2M receptor, or other component, can be achieved through covalent or non-covalent attachments. In one embodiment, the attachment may be indirect, *i.e.* through an attached antibody. In another embodiment, the  $\alpha$ 2M receptor and negative controls are tagged with an epitope, such as glutathione S-transferase (GST) so  
35 that the attachment to the solid surface can be mediated by a commercially available antibody such as anti-GST (Santa Cruz Biotechnology).

For example, such an affinity binding assay may be performed using a the  $\alpha 2M$  receptor which is immobilized to a solid support. Typically, the non-mobilized component of the binding reaction, in this case either ligand (e.g., HSP) or the test compound, is labeled to enable detection. A variety of labeling methods are available and may be used, such as  
5 luminescent, chromophore, fluorescent, or radioactive isotope or group containing same, and nonisotopic labels, such as enzymes or dyes. In a preferred embodiment, the test compound is labeled with a fluorophore such as fluorescein isothiocyanate (FITC, available from Sigma Chemicals, St. Louis).

The labeled test compounds, or ligand (e.g., HSP) plus test compounds, are then  
10 allowed to contact with the solid support, under conditions that allow specific binding to occur. After the binding reaction has taken place, unbound and non-specifically bound test compounds are separated by means of washing the surface. Attachment of the binding partner to the solid phase can be accomplished in various ways known to those skilled in the art, including but not limited to chemical cross-linking, non-specific adhesion to a plastic  
15 surface, interaction with an antibody attached to the solid phase, interaction between a ligand attached to the binding partner (such as biotin) and a ligand-binding protein (such as avidin or streptavidin) attached to the solid phase, and so on.

Finally, the label remaining on the solid surface may be detected by any detection method known in the art. For example, if the test compound is labeled with a fluorophore, a  
20 fluorimeter may be used to detect complexes.

Preferably, the  $\alpha 2M$  receptor is added to binding assays in the form of intact cells that express the  $\alpha 2M$  receptor, or isolated membranes containing the  $\alpha 2M$  receptor. Thus, direct binding to the  $\alpha 2M$  receptor or the ability of a test compound to modulate a ligand- $\alpha 2M$  receptor complex (e.g., HSP-  $\alpha 2M$  receptor complex) may be assayed in intact cells in  
25 culture or in animal models in the presence and absence of the test compound. A labeled ligand (e.g., HSP) may be mixed with cells that express the  $\alpha 2M$  receptor, or to crude extracts obtained from such cells, and the test compound may be added. Isolated membranes may be used to identify compounds that interact with the  $\alpha 2M$  receptor. For example, in a typical experiment using isolated membranes, cells may be genetically engineered to express  
30 the  $\alpha 2M$  receptor. Membranes can be harvested by standard techniques and used in an *in vitro* binding assay. Labeled ligand (e.g.,  $^{125}I$ -labeled HSP) is bound to the membranes and assayed for specific activity; specific binding is determined by comparison with binding assays performed in the presence of excess unlabeled (cold) ligand. Alternatively, soluble  $\alpha 2M$  receptor may be recombinantly expressed and utilized in non-cell based assays to  
35 identify compounds that bind to the  $\alpha 2M$  receptor. The recombinantly expressed  $\alpha 2M$  receptor polypeptides or fusion proteins containing the extracellular domain (ECD) of the  $\alpha 2M$  receptor, or one or more subdomains thereof, can be used in the non-cell based

screening assays. Alternatively, peptides corresponding to one or more of the CDs of the  $\alpha$ 2M receptor, or fusion proteins containing one or more of the CDs of the  $\alpha$ 2M receptor can be used in non-cell based assay systems to identify compounds that bind to the cytoplasmic portion of the  $\alpha$ 2M receptor; such compounds may be useful to modulate the signal transduction pathway of the  $\alpha$ 2M receptor. In non-cell based assays the recombinantly expressed the  $\alpha$ 2M receptor is attached to a solid substrate such as a test tube, microtiter well or a column, by means well known to those in the art (see Ausubel *et al.*, *supra*). The test compounds are then assayed for their ability to bind to the  $\alpha$ 2M receptor.

Alternatively, the binding reaction may be carried out in solution. In this assay, the labeled component is allowed to interact with its binding partner(s) in solution. If the size differences between the labeled component and its binding partner(s) permit such a separation, the separation can be achieved by passing the products of the binding reaction through an ultrafilter whose pores allow passage of unbound labeled component but not of its binding partner(s) or of labeled component bound to its partner(s). Separation can also be achieved using any reagent capable of capturing a binding partner of the labeled component from solution, such as an antibody against the binding partner, a ligand-binding protein which can interact with a ligand previously attached to the binding partner, and so on.

In a one embodiment, for example, a phage library can be screened by passing phage from a continuous phage display library through a column containing purified  $\alpha$ 2M receptor, or derivative, analog, fragment, or domain, thereof, linked to a solid phase, such as plastic beads. By altering the stringency of the washing buffer, it is possible to enrich for phage that express peptides with high affinity for the  $\alpha$ 2M receptor. Phage isolated from the column can be cloned and the affinities of the short peptides can be measured directly. Sequences for more than one oligonucleotide can be combined to test for even higher affinity binding to the  $\alpha$ 2M receptor. Knowing which amino acid sequences confer the strongest binding to the  $\alpha$ 2M receptor, computer models can be used to identify the molecular contacts between the  $\alpha$ 2M receptor and the test compound. This will allow the design of non-protein compounds which mimic those contacts. Such a compound may have the same activity of the peptide and can be used therapeutically, having the advantage of being efficient and less costly to produce.

In another specific embodiment of this aspect of the invention, the solid support is membranes containing the  $\alpha$ 2M receptor attached to a microtiter dish. Test compounds, for example, cells that express library members are cultivated under conditions that allow expression of the library members in the microtiter dish. Library members that bind to the protein (or nucleic acid or derivative) are harvested. Such methods, are described by way of example in Parmley and Smith, 1988, *Gene* 73:305-318; Fowlkes *et al.*, 1992,

BioTechniques 13:422-427; PCT Publication No. WO 94/18318; and in references cited hereinabove.

In another embodiment of the present invention, interactions between the  $\alpha$ 2M receptor or ligand (e.g., HSP) and a test compound may be assayed *in vitro*. Known or unknown molecules are assayed for specific binding to the  $\alpha$ 2M receptor nucleic acids, proteins, or derivatives under conditions conducive to binding, and then molecules that specifically bind to the  $\alpha$ 2M receptor are identified. The two components can be measured in a variety of ways. One approach is to label one of the components with an easily detectable label, place it together with a test component(s) under conditions that allow binding to occur, perform a separation step which separates bound labeled component from unbound labeled component, and then measure the amount of bound component. In one embodiment, the  $\alpha$ 2M receptor can be labeled and added to a test agent, using conditions that allow binding to occur. Binding of the test agent can be determined using polyacrylamide gel analysis to compare complexes formed in the presence and absence of the test agent.

In yet another embodiment, binding of ligand (e.g., HSP) to the  $\alpha$ 2M receptor may be assayed in intact cells in animal models. A labeled ligand (e.g., HSP) may be administered directly to an animal, with and without a test compound. Uptake of the ligand (e.g., HSP) may be measured in the presence and the absence of test compound. For these assays, host cells to which the test compound is added may be genetically engineered to express the  $\alpha$ 2M receptor and/or ligand (e.g., HSP), which may be transient, induced or constitutive, or stable. For the purposes of the screening methods of the present invention, a wide variety of host cells may be used including, but not limited to, tissue culture cells, mammalian cells, yeast cells, and bacteria. Mammalian cells such as macrophages or other cells that express the  $\alpha$ 2M receptor, *i.e.*, cells of the monocytic lineage, liver parenchymal cells, fibroblasts, keratinocytes, neuronal cells, and placental syncytiotrophoblasts, may be a preferred cell type in which to carry out the assays of the present invention. Bacteria and yeast are relatively easy to cultivate but process proteins differently than mammalian cells.

### 5.2.2 $\alpha$ 2M RECEPTOR ACTIVITY ASSAYS

After identification of a test compound that interacts with, or modulates the interaction of a ligand (e.g., HSP) with  $\alpha$ 2MR, the test compound can be further characterized to measure its effect on  $\alpha$ 2MR activity and the ligand- $\alpha$ 2MR endocytic signaling pathway. For example, the test compound may be characterized by testing its effect on ligand (e.g., HSP) / $\alpha$ 2MR cellular activity *in vivo*. Such assays include downstream signaling assays, antigen presentation assays, assays for antigen-specific activation of cytotoxic T cells, and the like.

In various embodiments, a candidate compound identified in a primary assay may be tested for its effect on innate  $\alpha$ 2MR signaling activity. For example, downstream signaling effects of  $\alpha$ 2M receptor activation which can be assayed include, but are not limited to: enhanced locomotion and chemotaxis of macrophages (Forrester *et al.*, 1983, *Immunology* 50: 251-259), down regulation of proteinase synthesis, and elevation of intracellular calcium, inositol phosphates and cyclic AMP (Misra *et al.*, 1993, *Biochem. J.*, 290:885-891). Other innate immune responses that can be tested are release of cytokines (*i.e.*, IL-12, IL1 $\beta$ , GMCSF, and TNF $\alpha$ ). Thus, as secondary assays, any identified candidate compound can be tested for changes in such activities in the presence and absence.

For example, in one embodiment, a chemotaxis assay can be used to further characterize a candidate identified by a primary screening assay. It is known that  $\alpha$ 2M modified by protease interaction can induce directional migration of cells towards their ligand. A number of techniques can be used to test chemotactic migration *in vitro* (see, *e.g.*, Leonard *et al.*, 1995, "Measurement of  $\alpha$  and  $\beta$  Chemokines", in *Current Protocols in Immunology*, 6.12.1-6.12.28; Ed. Coligan *et al.*, John Wiley & Sons, Inc. 1995). For example, in one embodiment, a candidate compound can be tested for its ability to modulate the ability of  $\alpha$ 2MR to induce migration of cells that express the receptor using a chemokine gradient in a multiwell Boyden chemotaxis chamber. In a specific example of this method, a serial dilution of a ligand (*e.g.*, an HSP) /  $\alpha$ 2MR antagonist or agonist test compound identified in the primary screen is placed in the bottom wells of the Boyden chemotaxis chamber. A constant amount of ligand is also added to the dilution series. As a control, at least one aliquot contains only ligand (*e.g.*, HSP). The contribution of the antagonist or agonist compound to the chemotactic activity of  $\alpha$ 2MR is measured by comparing number of migrating cells on the lower surface of the membrane filter of the aliquots containing only ligand (*e.g.*, HSP), with the number of cells in aliquots containing test compound and ligand (*e.g.*, HSP). If addition of the test compound to the ligand (*e.g.*, HSP) solution results in a decrease in the number of cells detected the membrane relative to the number of cells detected using a solution containing only ligand (*e.g.*, HSP), then an antagonist of ligand (*e.g.*, HSP) induction of chemotactic activity of  $\alpha$ 2MR-expressing cells is identified.

Elevation in intracellular ionized calcium concentration ( $[Ca^{2+}]_i$ ) is also an indicator of  $\alpha$ 2MR activation (Misra *et al.*, 1993, *supra*). Thus, in another embodiment, calcium flux assays can be used as secondary screens to further characterize modulators of ligand- $\alpha$ 2MR interactions. Intracellular calcium ion concentration can be measured in cells that express the  $\alpha$ 2M receptor in the presence of the ligand, in the presence and the absence of a test compound. For example, calcium mobilization can be detected and measured by flow cytometry, by labeling with fluorescent dyes that are trapped intracellularly. A fluorescent dye such as Indo-1 exhibits a change in emission spectrum upon binding calcium, the ratio of

fluorescence produced by the calcium-bound dye to that produced by the unbound dye may be used to estimate the intracellular calcium concentration. In a specific embodiment, cells are incubated in a cuvette in media containing Indo-1 at 37°C and are excited, and fluorescence is measured using a fluorimeter (Photon Technology Corporation, International). The ligand is added at a specific time point, in the presence and the absence of a test compound, EGTA is added to the cuvette to release and chelate total calcium, and the response is measured. Binding of ligand results in increased intracellular Ca<sup>2+</sup> concentration in cells that express α2MR. An agonist results in a relative increased intracellular Ca<sup>2+</sup> concentration, whereas an antagonist results in a relative decreased intracellular Ca<sup>2+</sup> concentration

10 In other embodiments, antigen-specific response assays may be used to detect the effect of a candidate compound on presentation of antigenic molecule by an α2MR ligand, for example an HSP or HSP complex. For example, an antigen presentation assay may be performed to determine the effect of a compound *in vivo* on the uptake of complexes capable of interacting with the α2M receptor, *e.g.*, HSP-antigenic molecule complexes, by cells expressing the α2M receptor. Such re-presentation assays are known in the art, and have been described previously (Suto and Srivastava, 1995, Science 269:1585-1588). For example, in one embodiment, antigen presenting cells, such as a macrophage cell line (*e.g.*, RAW264.7), are mixed with antigen-specific T cells in media, using approximately 10,000 cells of each type at approximately a 1:1 ratio. Complexes of HSP (10 μg/ml) and a peptide antigen, as well as test compound, is added to the cells and the culture is incubated for approximately 20 hours. Stimulation of T cells may then be measured in the presence and absence of test compound.

In another embodiment, antigen-specific T cell stimulation may be assayed. In one embodiment an IFN-γ release assay may be used. After washing, cells are fixed, permeabilized, and reacted with dye-labeled antibodies reactive with human IFN-γ (PE- anti-IFN-γ). Samples are analyzed by flow cytometry using standard techniques. Alternatively, a filter immunoassay, ELISA (enzyme linked immunosorbent assay), or enzyme-linked immunospot assay (ELISPOT) assay, may be used to detect specific cytokines produced by an activated T cell. In one embodiment, for example, a nitrocellulose-backed microtiter plate is coated with a purified cytokine-specific primary antibody, *i.e.*, anti-IFN-γ, and the plate is blocked to avoid background due to nonspecific binding of other proteins. A sample of APC cells stimulated with antigen is diluted onto the wells of the microtiter plate. A labeled, *e.g.*, biotin-labeled, secondary anti-cytokine antibody is added. The antibody cytokine complex can then be detected, *i.e.*, by enzyme-conjugated streptavidin – cytokine-secreting cells will appear as “spots” by visual, microscopic, or electronic detection methods. In another embodiment, “tetramer staining” assay (Altman *et al.*, 1996, Science 274: 94-96) may be used to identify antigen-specific T-cells. For example, an MHC molecule containing a

specific peptide antigen, such as a tumor-specific antigen, is multimerized to make soluble peptide tetramers and labeled, for example, by complexing to streptavidin. The MHC-peptide antigen complex is then mixed with a population of stimulated T cells. Biotin is then used to stain T cells which recognize and bind to the MHC-antigen complex.

5

### 5.2.3 COMPOUNDS THAT CAN BE SCREENED IN ACCORDANCE WITH THE INVENTION

The screening assays described herein may be used to identify small molecules, peptides or proteins, or derivatives, analogs and fragments thereof, that interact with, or modulate the interaction of a ligand (*e.g.*, HSP) with the  $\alpha 2M$  receptor. The compounds which may be screened in accordance with the invention include, but are not limited to small molecules, peptides, antibodies and fragments thereof, and other organic compounds (*e.g.*, peptidomimetics) that bind to the ECD of the  $\alpha 2M$  receptor and either inhibit the activity triggered by the natural ligand (*i.e.*, antagonists) or mimic the activity triggered by the natural ligand (*i.e.*, agonists), as well as small molecules, peptides, antibodies or fragments thereof, and other organic compounds. In one embodiment, such compounds include sequences of the  $\alpha 2M$  receptor, such as the ECD of the  $\alpha 2M$  receptor (or a portion thereof), which can bind to and "neutralize" natural ligands, such as HSPs,  $\alpha 2M$ , LDL, *etc.* In another embodiment, such compounds include ligand sequences, such as HSP sequences and/or  $\alpha 2M$  sequences, which can bind to the active site of the  $\alpha 2M$  receptor, and block its activity.

Compounds that may be used for screening include, but are not limited to, peptides such as, for example, soluble peptides, including but not limited to members of random peptide libraries; (see, *e.g.*, Lam *et al.*, 1991, Nature 354:82-84; Houghten *et al.*, 1991, Nature 354:84-86), and combinatorial chemistry-derived molecular library made of D- and/or L- configuration amino acids, phosphopeptides (including, but not limited to, members of random or partially degenerate, directed phosphopeptide libraries; see, *e.g.*, Songyang *et al.*, 1993, Cell 72:767-778), antibodies (including, but not limited to, polyclonal, monoclonal, humanized, anti-idiotypic, chimeric or single chain antibodies, and FAb, F(ab')<sub>2</sub> and FAb expression library fragments, and epitope-binding fragments thereof), and small organic or inorganic molecules.

In one embodiment of the present invention, peptide libraries may be used as a source of test compounds that can be used to screen for modulators of  $\alpha 2MR$  interactions, such as HSP-  $\alpha 2M$  receptor. Diversity libraries, such as random or combinatorial peptide or nonpeptide libraries can be screened for molecules that specifically bind to the  $\alpha 2M$  receptor. Many libraries are known in the art that can be used, *e.g.*, chemically synthesized libraries, recombinant (*e.g.*, phage display libraries), and *in vitro* translation-based libraries.

Examples of chemically synthesized libraries are described in Fodor *et al.*, 1991, Science 251:767-773; Houghten *et al.*, 1991, Nature 354:84-86; Lam *et al.*, 1991, Nature 354:82-84; Medynski, 1994, Bio/Technology 12:709-710; Gallop *et al.*, 1994, J. Medicinal Chemistry 37(9):1233-1251; Ohlmeyer *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:10922-10926; Erb *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91:11422-11426; Houghten *et al.*, 1992, Biotechniques 13:412; Jayawickreme *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91:1614-1618; Salmon *et al.*, 1993, Proc. Natl. Acad. Sci. USA 90:11708-11712; PCT Publication No. WO 93/20242; and Brenner and Lerner, 1992, Proc. Natl. Acad. Sci. USA 89:5381-5383.

10 Examples of phage display libraries are described in Scott & Smith, 1990, Science 249:386-390; Devlin *et al.*, 1990, Science, 249:404-406; Christian *et al.*, 1992, J. Mol. Biol. 227:711-718; Lenstra, 1992, J. Immunol. Meth. 152:149-157; Kay *et al.*, 1993, Gene 128:59-65; and PCT Publication No. WO 94/18318 dated August 18, 1994.

By way of examples of nonpeptide libraries, a benzodiazepine library (*see e.g.*, Bunin *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91:4708-4712) can be adapted for use. Peptoid libraries (Simon *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:9367-9371) can also be used. Another example of a library that can be used, in which the amide functionalities in peptides have been permethylated to generate a chemically transformed combinatorial library, is described by Ostresh *et al.* (1994, Proc. Natl. Acad. Sci. USA 91:11138-11142).

20 Screening the libraries can be accomplished by any of a variety of commonly known methods. See, *e.g.*, the following references, which disclose screening of peptide libraries: Parmley & Smith, 1989, Adv. Exp. Med. Biol. 251:215-218; Scott & Smith, 1990, Science 249:386-390; Fowlkes *et al.*, 1992; BioTechniques 13:422-427; Oldenburg *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:5393-5397; Yu *et al.*, 1994, Cell 76:933-945; Staudt *et al.*, 1988, Science 241:577-580; Bock *et al.*, 1992, Nature 355:564-566; Tuerk *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:6988-6992; Ellington *et al.*, 1992, Nature 355:850-852; U.S. Patent No. 5,096,815, U.S. Patent No. 5,223,409, and U.S. Patent No. 5,198,346, all to Ladner *et al.*; Rebar & Pabo, 1993, Science 263:671-673; and PCT Publication No. WO 94/18318.

30 In another embodiment of the present invention, the screening may be performed by adding the labeled ligand (*e.g.*, HSP) to *in vitro* translation systems such as a rabbit reticulocyte lysate (RRL) system and then proceeding with *in vitro* priming reaction. *In vitro* translation-based libraries include but are not limited to those described in PCT Publication No. WO 91/05058 dated April 18, 1991; and Mattheakis *et al.*, 1994, Proc. Natl. Acad. Sci. USA 91:9022-9026.

Compounds that can be tested and identified methods described herein can include, but are not limited to, compounds obtained from any commercial source, including Aldrich

(Milwaukee, WI 53233), Sigma Chemical (St. Louis, MO), Fluka Chemie AG (Buchs, Switzerland) Fluka Chemical Corp. (Ronkonkoma, NY;), Eastman Chemical Company, Fine Chemicals (Kingsport, TN), Boehringer Mannheim GmbH (Mannheim, Germany), Takasago (Rockleigh, NJ), SST Corporation (Clifton, NJ), Ferro (Zachary, LA 70791), Riedel-deHaen  
5 Aktiengesellschaft (Seelze, Germany), PPG Industries Inc., Fine Chemicals (Pittsburgh, PA 15272). Further any kind of natural products may be screened using the methods of the invention, including microbial, fungal, plant or animal extracts.

Furthermore, diversity libraries of test compounds, including small molecule test compounds, may be utilized. For example, libraries may be commercially obtained from  
10 Specs and BioSpecs B.V. (Rijswijk, The Netherlands), Chembridge Corporation (San Diego, CA), Contract Service Company (Dolgoprudny, Moscow Region, Russia), Comgenex USA Inc. (Princeton, NJ), Maybridge Chemicals Ltd. (Cornwall PL34 OHW, United Kingdom), and Asinex (Moscow, Russia).

Still further, combinatorial library methods known in the art, can be utilize, including,  
15 but not limited to: biological libraries; spatially addressable parallel solid phase or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of  
20 compounds (Lam, 1997, *Anticancer Drug Des.* 12:145). Combinatorial libraries of test compounds, including small molecule test compounds, can be utilized, and may, for example, be generated as disclosed in Eichler & Houghten, 1995, *Mol. Med. Today* 1:174-180; Dolle, 1997, *Mol. Divers.* 2:223-236; and Lam, 1997, *Anticancer Drug Des.* 12:145-167.

Examples of methods for the synthesis of molecular libraries can be found in the art,  
25 for example in: DeWitt *et al.*, 1993, *Proc. Natl. Acad. Sci. USA* 90:6909; Erb *et al.*, 1994, *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann *et al.*, 1994, *J. Med. Chem.* 37:2678; Cho *et al.*, 1993, *Science* 261:1303; Carrell *et al.*, 1994, *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell *et al.*, 1994, *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop *et al.*, 1994, *J. Med. Chem.* 37:1233.  
30

Libraries of compounds may be presented in solution (*e.g.*, Houghten, 1992, *BioTechniques* 13:412-421), or on beads (Lam, 1991, *Nature* 354:82-84), chips (Fodor, 1993, *Nature* 364:555-556), bacteria (U.S. Patent No. 5,223,409), spores (Patent Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* 89:1865-1869) or phage (Scott and Smith, 1990, *Science* 249:386-390; Devlin, 1990, *Science* 249:404-406; Cwirla *et al.*, 1990, *Proc. Natl. Acad. Sci. USA* 87:6378-6382; and Felici, 1991, *J. Mol. Biol.* 222:301-310).  
35

Screening the libraries can be accomplished by any of a variety of commonly known methods. See, *e.g.*, the following references, which disclose screening of peptide libraries: Parmley & Smith, 1989, *Adv. Exp. Med. Biol.* 251:215-218; Scott & Smith, 1990, *Science* 249:386-390; Fowlkes *et al.*, 1992; *BioTechniques* 13:422-427; Oldenburg *et al.*, 5 1992, *Proc. Natl. Acad. Sci. USA* 89:5393-5397; Yu *et al.*, 1994, *Cell* 76:933-945; Staudt *et al.*, 1988, *Science* 241:577-580; Bock *et al.*, 1992, *Nature* 355:564-566; Tuerk *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* 89:6988-6992; Ellington *et al.*, 1992, *Nature* 355:850-852; U.S. Patent No. 5,096,815, U.S. Patent No. 5,223,409, and U.S. Patent No. 5,198,346, all to Ladner *et al.*; Rebar & Pabo, 1993, *Science* 263:671-673; and PCT Publication No. 10 WO 94/18318.

### 5.3 IDENTIFICATION OF FRAGMENTS OF THE $\alpha$ 2M RECEPTOR AND/OR $\alpha$ 2M RECEPTOR LIGANDS, SUCH AS HSPTS, USEFUL FOR IMMUNOTHERAPY

The invention also encompasses methods for identifying ligand-binding  $\alpha$ 2MR 15 fragments (such as "HSP-binding domains"), and analogs, muteins, or derivatives thereof, which are capable of binding to, and uptake of,  $\alpha$ 2MR ligand-antigenic peptide, such as HSP-antigenic peptide complexes. Such ligand-binding  $\alpha$ 2MR fragment, *e.g.*, HSP-binding domains, can then be tested for activity *in vivo* and *in vitro* using the  $\alpha$ 2M receptor/ligand binding assays, described in Section 5.2.1, above. In one embodiment, such a method for 20 identifying an  $\alpha$ 2MR fragment capable of binding a heat shock protein comprises the steps of: (a) contacting a heat shock protein with one or more  $\alpha$ 2MR fragments; and (b) identifying an  $\alpha$ 2MR polypeptide fragment which specifically binds to the heat shock protein.

Ligand-binding domains, *e.g.*, HSP-binding domains, of the  $\alpha$ 2MR capable of 25 binding ligand-antigenic peptide complexes, such as HSP-antigenic peptide complexes, and can be further tested for activity using either *in vivo* binding assays, re-presentation assays, or CTL assays, such as those described in Section 5.2.2, above. For example, one such method for identifying an  $\alpha$ 2MR fragment capable of inducing an HSP- $\alpha$ 2M receptor-mediated process comprises the steps of: (a) contacting a heat shock protein with cell expressing 30  $\alpha$ 2MR fragment; and (b) measuring the level of  $\alpha$ 2MR activity in the cell, such that if the level of the HSP- $\alpha$ 2M receptor-mediated process or activity measured in (b) is greater than the level of  $\alpha$ 2MR activity in the absence of the  $\alpha$ 2MR fragment, then an  $\alpha$ 2MR fragment capable of inducing an HSP- $\alpha$ 2M receptor-mediated process is identified. Depending on 35 their behavior in such assays, such molecules can be used to either enhance or, alternatively, block the function of the receptor when administered or expressed *in vivo*. For example, these assays can be used to identify  $\alpha$ 2MR HSP-binding domains which can bind HSP-

antigen complexes and negatively interfere with their uptake by antigen presenting cells. These antagonists could be used to downregulate immune responses which are caused by cellular release of HSPs. Alternatively, certain  $\alpha 2$ MR HSP-binding domains may be used to enhance HSP-antigen complex uptake and signaling. Such agonists could be administered or expressed in subjects to elicit an immune response against an antigen of interest.

In another embodiment, the invention encompasses methods for identifying ligand fragment, such as HSP fragments, which are capable of binding and being taken up by the  $\alpha 2$ M receptor (“ $\alpha 2$ M receptor-binding domains”), and analogs, muteins, or derivatives thereof. As described for assays for  $\alpha 2$ M receptor-related polypeptides described above, such  $\alpha 2$ M receptor-binding domains can then be tested for activity *in vivo* and *in vitro* using the binding assays described in Section 5.2.1, above. For example, one such method for identifying a heat shock protein fragment capable of binding an  $\alpha 2$ M receptor comprises: (a) contacting an  $\alpha 2$ M receptor with one or more heat shock protein fragments; and (b) identifying a heat shock protein fragment which specifically binds to the  $\alpha 2$ M receptor.

Ligand fragments, such as HSP fragments, of interest may be further tested in cells, using *in vivo* binding assays, re-presentation assays, or CTL assays, such as those described in Section 5.2.2, above. For example, in one embodiment, such a method for identifying a heat shock protein fragment capable of inducing an HSP- $\alpha 2$ M receptor-mediated process comprises: a) contacting an  $\alpha 2$ M receptor fragment with a cell expressing a heat shock protein; and b) measuring the level of  $\alpha 2$ MR activity in the cell, such that if the level of the HSP- $\alpha 2$ M receptor-mediated process or activity measured in (b) is greater than the level of  $\alpha 2$ MR activity in the absence of said heat shock protein fragment. Alternatively,  $\alpha 2$ M receptor-binding domains which decrease uptake of HSPs could be used to block HSP uptake by the  $\alpha 2$ M receptor. In one embodiment, such HSP fragments comprising  $\alpha 2$ M receptor-binding domain sequences could be used to construct recombinant fusion proteins, comprised of a heat shock protein  $\alpha 2$ M receptor-binding domain and an antigenic peptide sequence. Such recombinant fusion proteins may be used to elicit an immune response and to treat or prevent immune diseases and disorders (Suzue *et al.*, 1997, Proc. Natl. Acad. Sci. U.S.A. 94: 13146-51).

The  $\alpha 2$ M receptor fragments, analogs, muteins, and derivatives and/or ligand (*e.g.*, HSP) fragments, analogs, muteins, and derivatives of the invention may be produced by recombinant DNA techniques, synthetic methods, or by enzymatic or chemical cleavage of native  $\alpha 2$ M receptor and/or ligands (*e.g.*, HSPs).

Any eukaryotic cell may serve as the nucleic acid source for obtaining the coding region of an  $\alpha 2$ M receptor or  $\alpha 2$ M receptor ligand (*e.g.*, HSP) gene. Nucleic acid sequences encoding ligand, *e.g.*, HSPs, and or the  $\alpha 2$ M receptor can be isolated from vertebrate, mammalian, as well as primate sources, including humans. Amino acid sequences and

nucleotide sequences of naturally occurring ligands, *e.g.*, HSPs, and  $\alpha$ 2M receptor are generally available in sequence databases, such as Genbank.

The DNA may be obtained by standard procedures known in the art by DNA amplification or molecular cloning directly from a tissue, cell culture, or cloned DNA (*e.g.*, a DNA "library"). Clones derived from genomic DNA may contain regulatory and intron DNA regions in addition to coding regions; clones derived from cDNA will contain only exon sequences. In a preferred embodiment, DNA can be amplified from genomic or cDNA by polymerase chain reaction (PCR) amplification using primers designed from the known sequence of an  $\alpha$ 2M receptor ligand, *e.g.*, HSP,  $\alpha$ 2M, or other  $\alpha$ 2MR ligand. The polymerase chain reaction (PCR) is commonly used for obtaining genes or gene fragments of interest. For example, a nucleotide sequence encoding a fragment of any desired length can be generated using PCR primers that flank the nucleotide sequence encoding the peptide-binding domain. Alternatively, an  $\alpha$ 2MR ligand, *e.g.*, HSP,  $\alpha$ 2M, or other  $\alpha$ 2MR ligand receptor gene sequence can be cleaved at appropriate sites with restriction endonuclease(s) if such sites are available, releasing a fragment of DNA encoding the peptide-binding domain. If convenient restriction sites are not available, they may be created in the appropriate positions by site-directed mutagenesis and/or DNA amplification methods known in the art (see, for example, Shankarappa *et al.*, 1992, PCR Method Appl. 1:277-278). The DNA fragment that encodes a fragment of the ligand (*e.g.*, HSP) or  $\alpha$ 2M receptor gene is then isolated, and ligated into an appropriate expression vector, care being taken to ensure that the proper translation reading frame is maintained. Alternatives to isolating the genomic DNA include, but are not limited to, chemically synthesizing the gene sequence itself from a known sequence or making cDNA to the mRNA which encodes the ligand (*e.g.*, HSP) and/or  $\alpha$ 2M receptor.

Any technique for mutagenesis known in the art can be used to modify individual nucleotides in a DNA sequence, for purpose of making amino acid substitution(s) in the expressed peptide sequence, or for creating/deleting restriction sites to facilitate further manipulations. Such techniques include but are not limited to, chemical mutagenesis, *in vitro* site-directed mutagenesis (Hutchinson, C., *et al.*, 1978, J. Biol. Chem 253:6551), oligonucleotide-directed mutagenesis (Smith, 1985, Ann. Rev. Genet. 19:423-463; Hill *et al.*, 1987, Methods Enzymol. 155:558-568), PCR-based overlap extension (Ho *et al.*, 1989, Gene 77:51-59), PCR-based megaprimer mutagenesis (Sarkar *et al.*, 1990, Biotechniques, 8:404-407), etc. Modifications can be confirmed by double stranded dideoxy DNA sequencing.

An alternative to producing  $\alpha$ 2M receptor and/or ligand (*e.g.*, HSP) fragments by recombinant techniques is peptide synthesis. For example, a peptide corresponding to a portion of an  $\alpha$ 2M receptor and/or ligand (*e.g.*, HSP) comprising the substrate-binding domain, or which binds peptides *in vitro*, can be synthesized by use of a peptide synthesizer.

Conventional peptide synthesis may be used or other synthetic protocols well known in the art.

In addition, analogs and derivatives of  $\alpha 2M$  receptor and/or ligand (*e.g.*, HSP) can be chemically synthesized. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the  $\alpha 2M$  receptor and/or ligand (*e.g.*, HSP) sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu,  $\epsilon$ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids such as  $\beta$ -methyl amino acids, C $\alpha$ -methyl amino acids, N $\alpha$ -methyl amino acids, and amino acid analogs in general.

$\alpha 2M$  receptor and/or ligand (*e.g.*, HSP) peptides, or a mutant or derivative thereof, may be synthesized by solid-phase peptide synthesis using procedures similar to those described by Merrifield, 1963, J. Am. Chem. Soc., 85:2149. During synthesis, N- $\alpha$ -protected amino acids having protected side chains are added stepwise to a growing polypeptide chain linked by its C-terminal and to an insoluble polymeric support *i.e.*, polystyrene beads. The peptides are synthesized by linking an amino group of an N- $\alpha$ -deprotected amino acid to an  $\alpha$ -carboxyl group of an N- $\alpha$ -protected amino acid that has been activated by reacting it with a reagent such as dicyclohexylcarbodiimide. The attachment of a free amino group to the activated carboxyl leads to peptide bond formation. The most commonly used N- $\alpha$ -protecting groups include Boc which is acid labile and Fmoc which is base labile. Details of appropriate chemistries, resins, protecting groups, protected amino acids and reagents are well known in the art and so are not discussed in detail herein (*See*, Atherton, *et al.*, 1989, Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, and Bodanszky, 1993, Peptide Chemistry, A Practical Textbook, 2nd Ed., Springer-Verlag).

Purification of the resulting fragment is accomplished using conventional procedures, such as preparative HPLC using gel permeation, partition and/or ion exchange chromatography. The choice of appropriate matrices and buffers are well known in the art and so are not described in detail herein.

In an alternative embodiment, fragments of an  $\alpha 2M$  receptor and/or ligand (*e.g.*, HSP) may be obtained by chemical or enzymatic cleavage of native or recombinant  $\alpha 2M$  receptor and/or ligand (*e.g.*, HSP) molecules. Specific chemical cleavage can be performed by cyanogen bromide, NaBH<sub>4</sub>, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, *etc.*. Endoproteases that cleave at specific sites can also be used. Such proteases are known in the art, including, but not limited to, trypsin,  $\alpha$ -chymotrypsin, V8 protease, papain, and proteinase K (see Ausubel *et al.*, (eds.), in

“Current Protocols in Molecular Biology”, Greene Publishing Associates and Wiley Interscience, New York, 17.4.6-17.4.8). The  $\alpha$ 2M receptor and/or ligand (*e.g.*, HSP) amino acid sequence of interest can be examined for the recognition sites of these proteases. An enzyme is chosen which can release a peptide-binding domain or peptide-binding fragment.

5 The  $\alpha$ 2M receptor and/or ligand (*e.g.*, HSP) molecule is then incubated with the protease, under conditions that allow digestion by the protease and release of the specifically designated peptide-binding fragments. Alternatively, such protease digestions can be carried out blindly, *i.e.*, not knowing which digestion product will contain the peptide-binding domain, using specific or general specificity proteases, such as proteinase K or pronase.

10 Once a fragment is prepared, the digestion products may be purified as described above, and subsequently tested for the ability to bind peptide or for immunogenicity. Methods for determining the immunogenicity of  $\alpha$ 2M receptor ligand (*e.g.*, HSP) complexes by cytotoxicity tests are described in Section 5.2.2.

#### 15 5.4 DRUG DESIGN

Upon identification of a compound that interacts with  $\alpha$ 2MR, or modulates the interaction of an  $\alpha$ 2M receptor ligand, such as an HSP, with the  $\alpha$ 2M receptor, such a compound can be further investigated to test for an ability to alter the immune response. In particular, for example, the compounds identified via the present methods can be further  
20 tested *in vivo* in accepted animal models of HSP- $\alpha$ 2MR-mediated processes and HSP-  $\alpha$ 2MR related disorders, such as, *e.g.*, immune disorders, proliferative disorders, and infectious diseases.

Computer modeling and searching technologies permit identification of compounds, or the improvement of already identified compounds, which can modulate the interaction of  
25 the  $\alpha$ 2M receptor with its ligand, *e.g.*, an HSP. Having identified such a compound or composition, the active sites or regions are identified. Such active sites might typically be ligand binding sites. The active site can be identified using methods known in the art including, for example, from the amino acid sequences of peptides, from the nucleotide sequences of nucleic acids, or from study of complexes of the relevant compound or  
30 composition with its natural ligand. In the latter case, chemical or X-ray crystallographic methods can be used to find the active site by finding where on the factor the complexed ligand is found.

Next, the three dimensional geometric structure of the active site is determined. This can be done by known methods, including X-ray crystallography, which can determine a  
35 complete molecular structure. On the other hand, solid or liquid phase NMR can be used to determine certain intra-molecular distances. Any other experimental method of structure

determination can be used to obtain partial or complete geometric structures. The geometric structures may be measured with a complexed ligand, natural or artificial, which may increase the accuracy of the active site structure determined.

If an incomplete or insufficiently accurate structure is determined, the methods of computer based numerical modeling can be used to complete the structure or improve its accuracy. Any recognized modeling method may be used, including parameterized models specific to particular biopolymers such as proteins or nucleic acids, molecular dynamics models based on computing molecular motions, statistical mechanics models based on thermal ensembles, or combined models. For most types of models, standard molecular force fields, representing the forces between constituent atoms and groups, are necessary, and can be selected from force fields known in physical chemistry. The incomplete or less accurate experimental structures can serve as constraints on the complete and more accurate structures computed by these modeling methods.

Finally, having determined the structure of the active site, either experimentally, by modeling, or by a combination, candidate modulating compounds can be identified by searching databases containing compounds along with information on their molecular structure. Such a search seeks compounds having structures that match the determined active site structure and that interact with the groups defining the active site. Such a search can be manual, but is preferably computer assisted. These compounds found from this search are potential the  $\alpha$ 2M receptor-modulating compounds.

Alternatively, these methods can be used to identify improved modulating compounds from an already known modulating compound or ligand. The composition of the known compound can be modified and the structural effects of modification can be determined using the experimental and computer modeling methods described above applied to the new composition. The altered structure is then compared to the active site structure of the compound to determine if an improved fit or interaction results. In this manner systematic variations in composition, such as by varying side groups, can be quickly evaluated to obtain modified modulating compounds or ligands of improved specificity or activity.

Further experimental and computer modeling methods useful to identify modulating compounds based upon identification of the active sites of either the  $\alpha$ 2M receptor or the HSP, and other  $\alpha$ 2M receptor ligands and their analogs, will be apparent to those of skill in the art.

Examples of molecular modeling systems are the CHARMM and QUANTA programs (Polygen Corporation, Waltham, MA). CHARMM performs the energy minimization and molecular dynamics functions. QUANTA performs the construction, graphic modelling and analysis of molecular structure. QUANTA allows interactive

construction, modification, visualization, and analysis of the behavior of molecules with each other.

A number of articles review computer modeling of drugs interactive with specific proteins, such as Rotivinen *et al.*) 1988, *Acta Pharmaceutical Fennica* 97:159-166); Ripka  
5 (1988 *New Scientist* 54-57); McKinally and Rossmann (1989, *Annu. Rev. Pharmacol. Toxicol.* 29:111-122); Perry and Davies, *OSAR: Quantitative Structure-Activity Relationships in Drug Design* pp. 189-193 Alan R. Liss, Inc. 1989; Lewis and Dean (1989, *Proc. R. Soc. Lond.* 236:125-140 and 141-162); and, with respect to a model receptor for nucleic acid components, Askew *et al.* (1989, *J. Am. Chem. Soc.* 111:1082-1090). Other  
10 computer programs that screen and graphically depict chemicals are available from companies such as BioDesign, Inc. (Pasadena, CA.), Allelix, Inc. (Mississauga, Ontario, Canada), and Hypercube, Inc. (Cambridge, Ontario). Although these are primarily designed for application to drugs specific to particular proteins, they can be adapted to design of drugs specific to regions of DNA or RNA, once that region is identified.

15

## 5.5 DIAGNOSTIC USES

The  $\alpha 2M$  receptor is a cell surface protein present on many tissues and cell types (Herz *et al.*, 1988, *EMBO J.* 7:4119-27; Moestrup *et al.*, 1992, *Cell Tissue Res.* 269: 375-82), that appears to be involved in the specific uptake and re-presentation of  $\alpha 2M$  receptor  
20 ligands, such as HSPs and HSP- peptide complexes. The  $\alpha 2M$  receptor was initially identified as a heat shock protein receptor due to its interaction with gp96, which is exclusively intracellular and is released as a result of necrotic but not apoptotic cell death. Thus, gp96 uptake by the  $\alpha 2M$  receptor may act as a sensor of necrotic cell death. As such,  $\alpha 2M$  receptor-ligand complexes may be used to detect and diagnose proliferative disorders,  
25 such as cancer, autoimmune disorders and infectious disease. Therefore,  $\alpha 2M$  receptor proteins, analogues, derivatives, and subsequences thereof,  $\alpha 2M$  receptor nucleic acids (and sequences complementary thereto), and anti- $\alpha 2M$  receptor antibodies, have uses in detecting and diagnosing such disorders.

The  $\alpha 2M$  receptor and  $\alpha 2M$  receptor nucleic acids can be used in assays to detect,  
30 prognose, or diagnose immune system disorders that may result in tumorigenesis, carcinomas, adenomas etc, and viral disease.

The molecules of the present invention can be used in assays, such as immunoassays, to detect, prognose, diagnose, or monitor various conditions, diseases, and disorders affecting  $\alpha 2M$  receptor expression, or monitor the treatment thereof. In particular, such an  
35 immunoassay is carried out by a method comprising contacting a sample derived from a patient with an HSP- $\alpha 2M$  receptor specific antibody under conditions such that

immunospecific binding can occur, and detecting or measuring the amount of any immunospecific binding by the antibody. In a specific aspect, such binding of antibody, in tissue sections, can be used to detect aberrant  $\alpha 2M$  receptor localization or aberrant (*e.g.*, low or absent) levels of  $\alpha 2M$  receptor. In a specific embodiment, antibody to the  $\alpha 2M$  receptor  
5 can be used to assay a patient tissue or serum sample for the presence of the  $\alpha 2M$  receptor where an aberrant level of  $\alpha 2M$  receptor is an indication of a diseased condition. By "aberrant levels," is meant increased or decreased levels relative to that present, or a standard level representing that present, in an analogous sample from a portion of the body or from a subject not having the disorder.

10 The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, immunohistochemistry radioimmunoassays, ELISA, "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent  
15 immunoassays, protein A immunoassays, to name but a few.

$\alpha 2M$  receptor genes and related nucleic acid sequences and subsequences, including complementary sequences, can also be used in hybridization assays.  $\alpha 2M$  receptor nucleic acid sequences, or subsequences thereof, comprising about at least 8 nucleotides, can be used as hybridization probes. Hybridization assays can be used to detect, prognose, diagnose, or  
20 monitor conditions, disorders, or disease states associated with aberrant changes in  $\alpha 2M$  receptor expression and/or activity as described *supra*. In particular, such a hybridization assay is carried out by a method comprising contacting a sample containing nucleic acid with a nucleic acid probe capable of hybridizing to  $\alpha 2M$  receptor DNA or RNA, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

25 In specific embodiments, diseases and disorders involving decreased immune responsiveness during an infection or malignant disorder can be diagnosed, or their suspected presence can be screened for, or a predisposition to develop such disorders can be detected, by detecting decreased levels of  $\alpha 2M$  receptor protein,  $\alpha 2M$  receptor RNA, or the  $\alpha 2M$  receptor functional activity (*e.g.*, binding to HSP, antibody-binding activity *etc.*), or by  
30 detecting mutations in  $\alpha 2M$  receptor RNA, DNA or  $\alpha 2M$  receptor protein (*e.g.*, translocations in the  $\alpha 2M$  receptor nucleic acids, truncations in the  $\alpha 2M$  receptor gene or protein, changes in nucleotide or amino acid sequence relative to wild-type  $\alpha 2M$  receptor) that cause decreased expression or activity of  $\alpha 2M$  receptor. Such diseases and disorders include but are not limited to those described in Sections 5.7, 5.8, and 5.9. By way of  
35 example, levels of the  $\alpha 2M$  receptor protein can be detected by immunoassay, levels of  $\alpha 2M$  receptor RNA can be detected by hybridization assays (*e.g.*, Northern blots, *in situ*-hybridization),  $\alpha 2M$  receptor activity can be assayed by measuring binding activities *in vivo*

or *in vitro*. Translocations, deletions, and point mutations in  $\alpha 2M$  receptor nucleic acids can be detected by Southern blotting, FISH, RFLP analysis, SSCP, PCR using primers, preferably primers that generate a fragment spanning at least most of the  $\alpha 2M$  receptor gene, sequencing of  $\alpha 2M$  receptor genomic DNA or cDNA obtained from the patient, *etc.*

5 In a preferred embodiment, levels of  $\alpha 2M$  receptor mRNA or protein in a patient sample are detected or measured relative to the levels present in an analogous sample from a subject not having the malignancy or hyperproliferative disorder. Decreased levels indicate that the subject may develop, or have a predisposition to developing, viral infection, malignancy, or hyperproliferative disorder.

10 In another specific embodiment, diseases and disorders involving a deficient immune responsiveness resulting in cell proliferation or in which cell proliferation is desirable for treatment, are diagnosed, or their suspected presence can be screened for, or a predisposition to develop such disorders can be detected, by detecting increased levels of the  $\alpha 2M$  receptor protein,  $\alpha 2M$  receptor RNA, or the  $\alpha 2M$  receptor functional activity (*e.g.*, HSP binding or  
15  $\alpha 2M$  receptor antibody, *etc.*), or by detecting mutations in  $\alpha 2M$  receptor RNA, DNA or protein (*e.g.*, translocations in  $\alpha 2M$  receptor nucleic acids, truncations in the gene or protein, changes in nucleotide or amino acid sequence relative to wild-type  $\alpha 2M$  receptor) that cause increased expression or activity of the  $\alpha 2M$  receptor. Such diseases and disorders include, but are not limited to, those described in Sections 5.7, 5.8, and 5.9. By way of example,  
20 levels of the  $\alpha 2M$  receptor protein, levels of  $\alpha 2M$  receptor RNA,  $\alpha 2M$  receptor binding activity, and the presence of translocations or point mutations can be determined as described above.

In a specific embodiment, levels of  $\alpha 2M$  receptor mRNA or protein in a patient sample are detected or measured, relative to the levels present in an analogous sample from a  
25 subject not having the disorder, in which increased levels indicate that the subject has, or has a predisposition to, an autoimmune disorder.

Kits for diagnostic use are also provided, that comprise in one or more containers an anti- $\alpha 2M$  receptor antibody, and, optionally, a labeled binding partner to the antibody. Alternatively, the anti- $\alpha 2M$  receptor antibody can be labeled (with a detectable marker, *e.g.*,  
30 a chemiluminescent, enzymatic, fluorescent, or radioactive moiety). A kit is also provided that comprises in one or more containers a nucleic acid probe capable of hybridizing to  $\alpha 2M$  receptor RNA. In a specific embodiment, a kit can comprise in one or more containers a pair of primers (*e.g.*, each in the size range of 6-30 nucleotides) that are capable of priming amplification [*e.g.*, by polymerase chain reaction (see *e.g.*, Innis *et al.*, 1990, PCR Protocols, Academic Press, Inc., San Diego, CA), ligase chain reaction (see EP 320,308) use of Q $\beta$   
35 replicase, cyclic probe reaction, or other methods known in the art] under appropriate reaction conditions of at least a portion of an  $\alpha 2M$  receptor nucleic acid. A kit can optionally

further comprise in a container a predetermined amount of a purified  $\alpha$ 2M receptor protein or nucleic acid, *e.g.*, for use as a standard or control.

## 5.6 THERAPEUTIC USES

5 The invention further encompasses methods for modulating the immune response. The  $\alpha$ 2M receptor recognizes and transports antigenic peptide complexes (*e.g.*, HSP-antigenic peptide complexes) for the purpose of presenting such antigenic molecules to cells of the immune system and eliciting an immune response. Thus, the compositions and methods of the invention may be used for therapeutic treatment of HSP- $\alpha$ 2M receptor-related  
10 disorders and conditions, such as autoimmune diseases, cancer and infectious diseases. In particular, as described in detail hereinbelow, recombinant cells comprising  $\alpha$ 2M receptor complexes, such as HSP-antigenic peptide complexes, antibodies and other compounds that interact with the  $\alpha$ 2M receptor, or modulate the interaction between the  $\alpha$ 2M receptor and its ligands, *e.g.*, HSP, as well as other compounds that modulate HSP- $\alpha$ 2M receptor-mediated  
15 processes may be used to elicit, or block, an immune response to treat such HSP-  $\alpha$ 2M receptor-related disorders and conditions.

### 5.6.1 THERAPEUTIC USE OF IDENTIFIED AGONISTS AND ANTAGONISTS

20 Compounds, such as those identified by screening methods provided herein, that interact with the  $\alpha$ 2M receptor (herein " $\alpha$ 2MR"), or modulate the interaction between the  $\alpha$ 2M receptor and its ligand, *e.g.*, HSP, can be useful as therapeutics. Such compounds, include, but are not limited to, agonists, antagonists, such as antibodies, antisense RNAs and ribozymes. Compounds which interfere with ligand (*e.g.*, HSP) - $\alpha$ 2M receptor interaction  
25 can be used to block an immune response, and can be used to treat autoimmune responses and conditions. Other antibodies, agonists, antagonists, antisense RNAs and ribozymes may upregulate ligand (*e.g.*, HSP)- $\alpha$ 2MR interaction, activity, or expression, and would enhance the uptake of antigen complexes (*e.g.*, HSP-antigen complexes), and therefore be useful in stimulating the host's immune system prior to, or concurrent with, the administration of a  
30 vaccine. Described below are methods and compositions for the use of such compounds in the treatment of HSP- $\alpha$ 2M receptor-related disorders, such as immune disorders, proliferative disorders, and infectious diseases.

In one embodiment an antagonist of  $\alpha$ 2M receptor-ligand (*e.g.*, HSP- $\alpha$ 2M receptor) interaction is used to block the immune response. Such antagonists include compounds that interfere with binding of a ligand (*e.g.*, an HSP) to the receptor by competing for binding to  
35 the  $\alpha$ 2M receptor, the ligand, or the ligand- $\alpha$ 2M receptor complex.

In one embodiment, the antagonist is an antibody specific for the  $\alpha 2M$  receptor, or a fragment thereof which contains the HSP ligand binding site. In another embodiment the antagonist is an antibody specific for an HSP, which interferes with binding of the HSP to the receptor.

5 In another embodiment, the antagonist is a peptide which comprises at least contiguous 10 amino acids of an HSP sequence. Such a peptide can bind to the ligand binding site of the  $\alpha 2M$  receptor to block the interaction of an HSP or HSP complex. In another embodiment, the antagonist is a peptide which comprises at least contiguous 10 amino acids of  $\alpha 2M$  sequence, which, like an HSP, can bind to the  $\alpha 2M$  receptor and  
10 interfere with the binding and uptake of HSP-antigen complexes. In yet another embodiment, the antagonist is a peptide which comprises at least contiguous 10 amino acids of  $\alpha 2M$  receptor sequence, in particular the ECD of the  $\alpha 2M$  receptor (or a portion thereof), which can bind to and "neutralize" natural ligands, such as HSPs,  $\alpha 2M$ , LDL, *etc.*

Such peptides may be produced synthetically or by using standard molecular biology  
15 techniques. Amino acid sequences and nucleotide sequences of naturally occurring  $\alpha 2M$  receptor ligands, such as  $\alpha 2M$  and HSPs, are generally available in sequence databases, such as GenBank. Computer programs, such as Entrez, can be used to browse the database, and retrieve any amino acid sequence and genetic sequence data of interest by accession number. Methods for recombinant and synthetic production of such peptides are described in Sections  
20 5.1.1 and 5.1.2.

Additionally, compounds, such as those identified via techniques such as those described hereinabove, in Section 5.2, that are capable of modulating  $\alpha 2M$  receptor gene product activity can be administered using standard techniques that are well known to those of skill in the art.

25

#### 5.6.1.1 COMPETITIVE ANTAGONISTS OF $\alpha 2MR$ -LIGAND INTERACTIONS

In one embodiment an antagonist of an  $\alpha 2MR$ -ligand (*e.g.*, HSP-  $\alpha 2M$  receptor) interaction is used to block the immune response to an antigen complex, *e.g.*, to treat an auto-  
30 immune disorder. Such antagonists include molecules that interfere with binding by binding to the  $\alpha 2M$  receptor, thereby interfering with binding of a ligand (*e.g.*, HSP) to the receptor. An example of this type of competitive inhibitor is an antibody to  $\alpha 2M$  receptor, or a fragment of  $\alpha 2MR$  which contains an HSP ligand binding site. Another example of a competitive antagonist is  $\alpha 2M$ , or a receptor-binding fragment thereof, which itself binds to  
35  $\alpha 2MR$ , thereby blocking the binding and uptake of HSP-antigen complexes by the cell.

An  $\alpha 2MR$ -ligand (*e.g.*, HSP) competitive inhibitor can be any type of molecule, including but not limited to a protein, nucleic acid or drug. In a preferred embodiment, an

HSP- $\alpha$ 2M competitive inhibitor is an  $\alpha$ 2MR-binding or an HSP-binding peptide. Examples of such peptides are provided below.

#### 5.6.1.1.1 $\alpha$ 2M RECEPTOR-BINDING PEPTIDES

##### 5 $\alpha$ Macroglobulin peptides

In one embodiment of the present invention, an HSP- $\alpha$ 2MR competitive antagonist is an  $\alpha$  macroglobulin, preferably  $\alpha$ 2M, or  $\alpha$ 2MR-binding portion thereof.

Functional expression of  $\alpha$ 2M or  $\alpha$ 2MR-binding portions thereof (including recombinant expression as a FX fusion protein, processing, purification and refolding) is preferably carried out as described by Holtet *et al.*, 1994, FEBS Lett. 344:242-246.

In a specific mode of the embodiment, an  $\alpha$ 2MR-binding portion of  $\alpha$ 2M consists of or comprises a fragment of the  $\alpha$ 2M RBD consisting of at least 10 (continuous) amino acids. In other modes of the embodiment, the fragment consists of at least 20, 30, 40, 50, 75 or 100 amino acids of the RBD. In specific modes of the embodiment, such fragments are not larger than 27, 138 or 153 amino acids. Most preferred peptides comprise one or both of amino acids Lys<sub>1370</sub> and Lys<sub>1374</sub>. Such peptides include those consisting of amino acids 1299-1451 (vRBD in FIG. 13B) (SEQ ID NO:8), 1314-1451 (SEQ ID NO:9) (RBD in FIG. 13B) or 1366-1392 (SEQ ID NO:10) of the mature  $\alpha$ 2M protein. Other preferred peptides include but are not limited to those consisting of amino acids 1300-1425 (SEQ ID NO:11), 1300-1400 (SEQ ID NO:12), 1300-1380 (SEQ ID NO:13), 1325-1425 (SEQ ID NO:14), 1325-1400 (SEQ ID NO:15), 1325-1380 (SEQ ID NO:16), 1350-1425 (SEQ ID NO:17), 1350-1400 (SEQ ID NO:18), or 1350-1380 (SEQ ID NO:19) of the mature human  $\alpha$ 2M protein.

Derivatives or analogs of  $\alpha$ 2M or  $\alpha$ 2MR-binding portions of  $\alpha$ 2M are also contemplated as competitive antagonists of HSP- $\alpha$ 2MR complexes. Such derivative or analogs include but are not limited to those molecules comprising regions that are substantially homologous to  $\alpha$ 2M, the  $\alpha$ 2M RBD or fragments thereof (*e.g.*, in various embodiments, at least 60% or 70% or 80% or 90% or 95% identity over an amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art) or whose encoding nucleic acid is capable of hybridizing to a coding  $\alpha$ 2M RBD sequence, under stringent, moderately stringent, or nonstringent conditions. In certain specific embodiments, an  $\alpha$ 2M derivative is a chimeric or fusion protein comprising an  $\alpha$ 2M protein or  $\alpha$ 2MR-binding portion thereof (preferably consisting of at least 10 amino acids of the  $\alpha$ 2M RBD comprising Lys<sub>1370</sub> and Lys<sub>1374</sub>) joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein.

In particular,  $\alpha$ 2M derivatives can be made by altering  $\alpha$ 2M coding sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due

to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as a  $\alpha$ 2M gene may be used in the practice of the present invention. These include but are not limited to nucleotide sequences comprising all or  $\alpha$ 2MR-binding portions of  $\alpha$ 2M genes which are altered by the substitution of different  
5 codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. Likewise, the  $\alpha$ 2M derivatives of the invention include, but are not limited to, those containing, as a primary amino acid sequence, all or an  $\alpha$ 2MR-binding portion of the amino acid sequence of an  $\alpha$ 2M protein, including altered sequences in which functionally equivalent amino acid residues are substituted for residues within the sequence  
10 resulting in a silent change. For example, one or more amino acid residues within the sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine,  
15 valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid.

The  $\alpha$ 2M derivatives and analogs of the invention can be produced by various  
20 methods known in the art. The manipulations which result in their production can occur at the gene or protein level. For example, the cloned  $\alpha$ 2M gene sequence can be modified by any of numerous strategies known in the art (Maniatis, T., 1990, Molecular Cloning, A Laboratory Manual, 2d ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York). The sequence can be cleaved at appropriate sites with restriction endonuclease(s),  
25 followed by further enzymatic modification if desired, isolated, and ligated *in vitro*. In the production of the gene encoding a derivative or analog of  $\alpha$ 2M, care should be taken to ensure that the modified gene remains within the same translational reading frame as  $\alpha$ 2M, uninterrupted by translational stop signals, in the gene region where the desired  $\alpha$ 2M activity is encoded.

30 Manipulations of the  $\alpha$ 2M sequence may also be made at the protein level. Included within the scope of the invention are  $\alpha$ 2M protein fragments or other derivatives or analogs which are differentially modified during or after translation, *e.g.*, by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of  
35 numerous chemical modifications may be carried out by known techniques, including but not limited to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain,

V8 protease, NaBH<sub>4</sub>; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

In addition, analogs and derivatives of  $\alpha 2M$  can be chemically synthesized. For example, an  $\alpha 2MR$ -binding portion of  $\alpha 2M$  can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the  $\alpha 2M$  sequence. Non-classical amino acids include but are not limited to the D-isomers of the common amino acids,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid,  $\gamma$ -Abu,  $\epsilon$ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine,  $\beta$ -alanine, fluoro-amino acids, designer amino acids such as  $\beta$ -methyl amino acids, C $\alpha$ -methyl amino acids, N $\alpha$ -methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

In other specific modes of the embodiment, an HSP- $\alpha 2MR$  competitive antagonist is another  $\alpha$  macroglobulin or  $\alpha 2MR$ -binding portion thereof, for example an  $\alpha$  macroglobulin RBD domain selected from Nielsen *et al.*, *supra*, Fig. 3, Group A.

#### *RAP*

In one embodiment of the present invention, an HSP- $\alpha 2MR$  competitive antagonist is  $\alpha 2MR$ -associated protein (RAP) (Genbank accession no. A39875) or an  $\alpha 2MR$ -binding portion thereof. In a specific mode of the embodiment, an  $\alpha 2MR$ -binding portion of RAP consists of or comprises a fragment of the RAP RBD consisting of at least 10 (continuous) amino acids. In other modes of the embodiment, the fragment consists of at least 20, 30, 40, 50, 75 or 100 amino acids of the RBD. In specific modes of the embodiment, such fragments are not larger than 28, 50 or 100 amino acids. In other specific modes of the embodiment, an  $\alpha 2MR$ -binding portion of RAP comprises an  $\alpha 2MR$ -binding portion of domain 1 or 3, *e.g.* as depicted in Nielsen *et al.*, *supra*, Fig. 3, Group D or E. Expression of recombinant RAP or an  $\alpha 2MR$ -binding portion thereof, *e.g.* domain 1 or 3, is preferably achieved as described by Andersen *et al.*, *supra*).

### 5.6.1.1.2 HSP-BINDING PEPTIDES

#### *$\alpha 2MR$ peptides*

In one embodiment of the present invention, an HSP- $\alpha 2MR$  competitive antagonist is  $\alpha 2MR$  peptide, preferably a soluble peptide, that can bind to HSPs and therefore competitively inhibit HSP binding to the native receptor.

Functional expression of HSP-binding portions of  $\alpha$ 2MR is preferably carried out as described for the CR8 domain by Huang *et al.*, 1999, J. Biol. Chem 274:14130-14136. Briefly, to maintain proper folding, the protein is expressed as a GST fusion, expressed recombinantly, the GST portion cleaved, uncleaved protein removed on GSH-Sepharose, and  
5 cleaved protein refolded. Since the complement repeats bind to calcium, proper folding is assayed by measuring the binding of the refolded protein to calcium.

In a specific mode of the embodiment, an HSP-binding portion of  $\alpha$ 2MR consists of or comprises at least one complement repeat, most preferably selected from CR3-CR10. In another specific mode of the embodiment, an HSP-binding portion of  $\alpha$ 2MR comprises a  
10 cluster of complement repeats, most preferably C1-II. In other modes of the embodiment, the HSP-binding portion consists of at least 10, more preferably at least 20, yet more preferably at least 30, yet more preferably at least 40, and most preferably at least 80 (continuous) amino acids. In specific modes of the embodiment, such fragments are not larger than 40-45 amino acids. In other specific modes of the embodiment, such fragments are not larger than  
15 80-90 amino acids. Exemplary preferred peptides include but are not limited to those consisting of amino acids 25-68 (SEQ ID NO:20), 25-110 (SEQ ID NO:21), 68-110 (SEQ ID NO:22), 853-894 (SEQ ID NO:23), 853-934 (SEQ ID NO:24), 853-974 (SEQ ID NO:25), 853-1013 (SEQ ID NO:26), 853-1060 (SEQ ID NO:27), 853-1102 (SEQ ID NO:28), 853-1183 (SEQ ID NO:29), 895-934 (SEQ ID NO:30), 895-974 (SEQ ID NO:31), 895-1013  
20 (SEQ ID NO:32), 895-1060 (SEQ ID NO:33), 895-1102 (SEQ ID NO:34), 895-1183 (SEQ ID NO:35), 935-974 (SEQ ID NO:36), 935-1013 (SEQ ID NO:37), 935-1060 (SEQ ID NO:38), 935-1102 (SEQ ID NO:39), 935-1183 (SEQ ID NO:40), 975-1013 (SEQ ID NO:41), 975-1060 (SEQ ID NO:42), 975-1143 (SEQ ID NO:43), 975-1183 (SEQ ID NO:44), 1014-1060 (SEQ ID NO:45), 1014-1102 (SEQ ID NO:46), 1014-1183 (SEQ ID NO:47), 1061-1102 (SEQ ID NO:48), 1061-1143 (SEQ ID NO:49), 1061-1183 (SEQ ID  
25 NO:50), 1103-1143 (SEQ ID NO:51), 1103-1183 (SEQ ID NO:52), or 1144-1183 (SEQ ID NO:53) of human  $\alpha$ 2MR.

Derivatives or analogs of HSP-binding portions  $\alpha$ 2MR also contemplated as competitive antagonists of HSP- $\alpha$ 2MR complexes. Such derivative or analogs include but  
30 are not limited to those molecules comprising regions that are substantially homologous to the extracellular domain of  $\alpha$ 2MR or fragments thereof (*e.g.*, in various embodiments, at least 60% or 70% or 80% or 90% or 95% identity over an amino acid sequence of identical size or when compared to an aligned sequence in which the alignment is done by a computer homology program known in the art) or whose encoding nucleic acid is capable of  
35 hybridizing to a sequence encoding an  $\alpha$ 2MR HSP-binding sequence, under stringent, moderately stringent, or nonstringent conditions. In certain specific embodiments, an  $\alpha$ 2MR derivative is a chimeric or fusion protein comprising an HSP-binding portion of  $\alpha$ 2MR,

preferably consisting of at least one complement repeat of C1-II) joined at its amino- or carboxy-terminus via a peptide bond to an amino acid sequence of a different protein. Such a chimeric protein can be produced recombinantly as described above, by omitting the cleavage repurification steps.

5 Other HSP-binding  $\alpha$ 2MR derivatives can be made by altering  $\alpha$ 2MR coding sequences by substitutions, additions or deletions that provide for functionally equivalent molecules. Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode substantially the same amino acid sequence as an HSP-binding  $\alpha$ 2MR gene or gene fragment may be used in the practice of the present invention. Selection of suitable  
10 alterations and production of HSP-binding  $\alpha$ 2MR derivatives can be made applying the same principles described above for  $\alpha$ 2M derivatives and using the general methods described in Sections 5.1.1 and 5.1.2.

#### *HSP peptides*

15 In another mode of the embodiment, the antagonist is a peptide which comprises at least contiguous 10 amino acids of an HSP sequence. Such a peptide can bind to the ligand binding site of the  $\alpha$ 2M receptor to block the interaction of an HSP or HSP complex.

Such peptides may be produced synthetically or by using standard molecular biology techniques. Amino acid sequences and nucleotide sequences of naturally occurring HSPs are  
20 generally available in sequence databases, such as GenBank. Computer programs, such as Entrez, can be used to browse the database, and retrieve any amino acid sequence and genetic sequence data of interest by accession number. Methods for recombinant and synthetic production of such peptides are described in Sections 5.1.1 and 5.1.2.

25 Additionally, compounds, such as those identified via techniques such as those described hereinabove, in Section 5.2, that are capable of modulating  $\alpha$ 2M receptor gene product activity can be administered using standard techniques that are well known to those of skill in the art.

#### 5.6.2 THERAPEUTIC USE OF THE $\alpha$ 2M RECEPTOR AGAINST CANCER AND INFECTIOUS DISEASES

30

In another embodiment, symptoms of certain  $\alpha$ 2M receptor gene disorders, such as autoimmune disorders, or proliferative or differentiative disorders causing tumorigenesis or cancer, may be ameliorated by modulating the level of  $\alpha$ 2M receptor gene expression and/or  $\alpha$ 2M receptor gene product activity. In one embodiment, for example, a decrease in  $\alpha$ 2M  
35 receptor gene expression may be useful to decrease  $\alpha$ 2M receptor activity, and ameliorate the symptoms of an autoimmune disorder. In this case, the level of  $\alpha$ 2M receptor gene expression may be decreased by using  $\alpha$ 2M receptor gene sequences in conjunction with

well-known antisense, gene "knock-out," ribozyme and/or triple helix methods. In another embodiment, an increase in  $\alpha 2M$  receptor gene expression may be desired to compensate for a mutant or impaired gene in an HSP- $\alpha 2M$  receptor-mediated pathway, and to ameliorate the symptoms of an HSP-  $\alpha 2M$  receptor-related disorder.

5           Among the compounds that may exhibit the ability to modulate the activity, expression or synthesis of the  $\alpha 2M$  receptor gene, including the ability to ameliorate the symptoms of an HSP- $\alpha 2M$  receptor related disorder are antisense, ribozyme, and triple helix molecules. Such molecules may be designed to reduce or inhibit either unimpaired, or if appropriate, mutant target gene activity. Techniques for the production and use of such  
10 molecules are well known to those of skill in the art.

          Antisense RNA and DNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing protein translation. Antisense approaches involve the design of oligonucleotides that are complementary to a target gene mRNA. The antisense oligonucleotides will bind to the complementary target gene mRNA transcripts and  
15 prevent translation. Absolute complementarity, although preferred, is not required.

          A sequence "complementary" to a portion of an RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to  
20 hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the longer the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex, as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

          In one embodiment, oligonucleotides complementary to non-coding regions of the  
25  $\alpha 2M$  receptor gene could be used in an antisense approach to inhibit translation of endogenous  $\alpha 2M$  receptor mRNA. Antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17  
30 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

          In an embodiment of the present invention, oligonucleotides complementary to the nucleic acids encoding the HSP receptor ligand binding domain are used.

          Regardless of the choice of target sequence, it is preferred that in vitro studies are first performed to quantitate the ability of the antisense oligonucleotide to inhibit gene  
35 expression. It is preferred that these studies utilize controls that distinguish between antisense gene inhibition and nonspecific biological effects of oligonucleotides. It is also preferred that these studies compare levels of the target RNA or protein with that of an internal control

RNA or protein. Additionally, it is envisioned that results obtained using the antisense oligonucleotide are compared with those obtained using a control oligonucleotide. It is preferred that the control oligonucleotide is of approximately the same length as the test oligonucleotide and that the nucleotide sequence of the oligonucleotide differs from the antisense sequence no more than is necessary to prevent specific hybridization to the target sequence.

The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, Proc. Natl. Acad. Sci. U.S.A. 86, 6553-6556; Lemaitre *et al.*, 1987, Proc. Natl. Acad. Sci. 84, 648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents (see, *e.g.*, Krol *et al.*, 1988, BioTechniques 6, 958-976) or intercalating agents (see, *e.g.*, Zon, 1988, Pharm. Res. 5, 539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including but not limited to 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including but not limited to arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group consisting of a phosphorothioate (S-

ODNs), a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate, a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

5 In yet another embodiment, the antisense oligonucleotide is an  $\alpha$ -anomeric oligonucleotide. An  $\alpha$ -anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual  $\beta$ -units, the strands run parallel to each other (Gautier *et al.*, 1987, Nucl. Acids Res. 15, 6625-6641). The oligonucleotide is a 2'-O-methylribonucleotide (Inoue *et al.*, 1987, Nucl. Acids Res. 15, 6131-6148), or a chimeric RNA-DNA analogue (Inoue *et al.*, 1987, FEBS Lett. 215, 327-330).

10 Oligonucleotides of the invention may be synthesized by standard methods known in the art, *e.g.* by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein *et al.* (1988, Nucl. Acids Res. 16, 3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer  
15 supports (Sarin *et al.*, 1988, Proc. Natl. Acad. Sci. U.S.A. 85, 7448-7451), etc.

While antisense nucleotides complementary to the target gene coding region sequence could be used, those complementary to the transcribed, untranslated region are most preferred.

In one embodiment of the present invention, gene expression downregulation is  
20 achieved because specific target mRNAs are digested by RNAse H after they have hybridized with the antisense phosphorothioate oligonucleotides (S-ODNs). Since no rules exist to predict which antisense S-ODNs will be more successful, the best strategy is completely empirical and consists of trying several antisense S-ODNs. Antisense phosphorothioate oligonucleotides (S-ODNs) will be designed to target specific regions of  
25 mRNAs of interest. Control S-ODNs consisting of scrambled sequences of the antisense S-ODNs will also be designed to assure identical nucleotide content and minimize differences potentially attributable to nucleic acid content. All S-ODNs can be synthesized by Oligos Etc. (Wilsonville, OR). In order to test the effectiveness of the antisense molecules when applied to cells in culture, such as assays for research purposes or *ex vivo* gene therapy  
30 protocols, cells will be grown to 60-80% confluence on 100 mm tissue culture plates, rinsed with PBS and overlaid with lipofection mix consisting of 8 ml Opti-MEM, 52.8  $\mu$ l Lipofectin, and a final concentration of 200 nM S-ODNs. Lipofections will be carried out using Lipofectin Reagent and Opti-MEM (Gibco BRL). Cells will be incubated in the presence of the lipofection mix for 5 hours. Following incubation the medium will be  
35 replaced with complete DMEM. Cells will be harvested at different time points post-lipofection and protein levels will be analyzed by Western blot.

Antisense molecules should be targeted to cells that express the target gene, either directly to the subject *in vivo* or to cells in culture, such as in *ex vivo* gene therapy protocols. A number of methods have been developed for delivering antisense DNA or RNA to cells; *e.g.*, antisense molecules can be injected directly into the tissue site, or modified antisense  
5 molecules, designed to target the desired cells (*e.g.*, antisense linked to peptides or antibodies that specifically bind receptors or antigens expressed on the target cell surface) can be administered systemically.

However, it is often difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation of endogenous mRNAs. Therefore a preferred approach  
10 utilizes a recombinant DNA construct in which the antisense oligonucleotide is placed under the control of a strong pol III or pol II promoter. The use of such a construct to transfect target cells in the patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the endogenous target gene transcripts and thereby prevent translation of the target gene mRNA. For example, a vector  
15 can be introduced *e.g.*, such that it is taken up by a cell and directs the transcription of an antisense RNA. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian  
20 cells. Expression of the sequence encoding the antisense RNA can be by any promoter known in the art to act in mammalian, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Bernoist and Chambon, 1981, Nature 290, 304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, 1980, Cell  
25 22, 787-797), the herpes thymidine kinase promoter (Wagner *et al.*, 1981, Proc. Natl. Acad. Sci. U.S.A. 78, 1441-1445), the regulatory sequences of the metallothionein gene (Brinster *et al.*, 1982, Nature 296, 39-42), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site. Alternatively, viral vectors can be used that selectively infect the desired tissue,  
30 in which case administration may be accomplished by another route (*e.g.*, systemically).

Ribozyme molecules designed to catalytically cleave target gene mRNA transcripts can also be used to prevent translation of target gene mRNA and, therefore, expression of target gene product. (See, *e.g.*, PCT International Publication WO90/11364, published October 4, 1990; Sarver *et al.*, 1990, Science 247, 1222-1225). In an embodiment of the  
35 present invention, oligonucleotides which hybridize to the HSP receptor gene are designed to be complementary to the nucleic acids encoding the HSP receptor ligand binding domain.

Ribozymes are enzymatic RNA molecules capable of catalyzing the specific cleavage of RNA. (For a review, see Rossi, 1994, *Current Biology* 4, 469-471). The mechanism of ribozyme action involves sequence specific hybridization of the ribozyme molecule to complementary target RNA, followed by an endonucleolytic cleavage event. The  
5 composition of ribozyme molecules must include one or more sequences complementary to the target gene mRNA, and must include the well known catalytic sequence responsible for mRNA cleavage. For this sequence, see, *e.g.*, U.S. Patent No. 5,093,246, which is incorporated herein by reference in its entirety.

While ribozymes that cleave mRNA at site specific recognition sequences can be  
10 used to destroy target gene mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in  
15 Myers, 1995, *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*, VCH Publishers, New York, (see especially fig. 4, p. 833) and in Haseloff & Gerlach, 1988, *Nature*, 334, 585-591, which is incorporated herein by reference in its entirety.

Preferably the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the target gene mRNA, *i.e.*, to increase efficiency and minimize the  
20 intracellular accumulation of non-functional mRNA transcripts.

The ribozymes of the present invention also include RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one that occurs naturally in *Tetrahymena thermophila* (known as the IVS, or L-19 IVS RNA) and that has been extensively described by Thomas Cech and collaborators (Zaug *et al.*, 1984, *Science*, 224, 574-578; Zaug and  
25 Cech, 1986, *Science*, 231, 470-475; Zaug *et al.*, 1986, *Nature*, 324, 429-433; published International patent application No. WO 88/04300 by University Patents Inc.; Been & Cech, 1986, *Cell*, 47, 207-216). The Cech-type ribozymes have an eight base pair active site which hybridizes to a target RNA sequence whereafter cleavage of the target RNA takes place. The invention encompasses those Cech-type ribozymes which target eight base-pair active site  
30 sequences that are present in the target gene.

As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (*e.g.*, for improved stability, targeting, etc.) and should be delivered to cells that express the target gene *in vivo*. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II  
35 promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous target gene messages and inhibit translation. Because ribozymes unlike

antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Endogenous target gene expression can also be reduced by inactivating or "knocking out" the target gene or its promoter using targeted homologous recombination (*e.g.*, see Smithies *et al.*, 1985, *Nature* 317, 230-234; Thomas & Capecchi, 1987, *Cell* 51, 503-512; Thompson *et al.*, 1989, *Cell* 5, 313-321; each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional target gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous target gene (either the coding regions or regulatory regions of the target gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express the target gene *in vivo*. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (*e.g.*, see Thomas & Capecchi, 1987 and Thompson, 1989, *supra*). However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors.

Alternatively, endogenous target gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target gene (i.e., the target gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the target gene in target cells in the body. (See generally, Helene, 1991, *Anticancer Drug Des.*, 6(6), 569-584; Helene *et al.*, 1992, *Ann. N.Y. Acad. Sci.*, 660, 27-36; and Maher, 1992, *Bioassays* 14(12), 807-815).

Nucleic acid molecules to be used in triple helix formation for the inhibition of transcription should be single stranded and composed of deoxyribonucleotides. The base composition of these oligonucleotides must be designed to promote triple helix formation via Hoogsteen base pairing rules, which generally require sizeable stretches of either purines or pyrimidines to be present on one strand of a duplex. Nucleotide sequences may be pyrimidine-based, which will result in TAT and CGC<sup>+</sup> triplets across the three associated strands of the resulting triple helix. The pyrimidine-rich molecules provide base complementarity to a purine-rich region of a single strand of the duplex in a parallel orientation to that strand. In addition, nucleic acid molecules may be chosen that are purine-rich, for example, contain a stretch of G residues. These molecules will form a triple helix with a DNA duplex that is rich in GC pairs, in which the majority of the purine residues are located on a single strand of the targeted duplex, resulting in GGC triplets across the three strands in the triplex.

Alternatively, the potential sequences that can be targeted for triple helix formation may be increased by creating a so called "switchback" nucleic acid molecule. Switchback molecules are synthesized in an alternating 5'-3', 3'-5' manner, such that they base pair with first one strand of a duplex and then the other, eliminating the necessity for a sizeable stretch of either purines or pyrimidines to be present on one strand of a duplex.

In instances wherein the antisense, ribozyme, and/or triple helix molecules described herein are utilized to inhibit mutant gene expression, it is possible that the technique may so efficiently reduce or inhibit the transcription (triple helix) and/or translation (antisense, ribozyme) of mRNA produced by normal target gene alleles that the possibility may arise wherein the concentration of normal target gene product present may be lower than is necessary for a normal phenotype. In such cases, to ensure that substantially normal levels of target gene activity are maintained, therefore, nucleic acid molecules that encode and express target gene polypeptides exhibiting normal target gene activity may, be introduced into cells via gene therapy methods such as those described, below, in Section 5.6.3 that do not contain sequences susceptible to whatever antisense, ribozyme, or triple helix treatments are being utilized. Alternatively, in instances whereby the target gene encodes an extracellular protein, it may be preferable to co-administer normal target gene protein in order to maintain the requisite level of target gene activity.

Anti-sense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules, as discussed above. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by *in vitro* and *in vivo* transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences may be incorporated into a wide variety of vectors that incorporate suitable RNA polymerase promoters such as the T7 or SP6 polymerase promoters. Alternatively, antisense cDNA constructs that synthesize antisense RNA constitutively or inducibly, depending on the promoter used, can be introduced stably into cell lines.

### 5.6.3 GENE REPLACEMENT THERAPY

With respect to an increase in the level of normal  $\alpha 2M$  receptor gene expression and/or  $\alpha 2M$  receptor gene product activity,  $\alpha 2M$  receptor gene nucleic acid sequences can, for example, be utilized for the treatment of immune disorders resulting in proliferative disorders such as cancer. Such treatment can be administered, for example, in the form of gene replacement therapy. Specifically, one or more copies of a normal  $\alpha 2M$  receptor gene or a portion of the  $\alpha 2M$  receptor gene that directs the production of an  $\alpha 2M$  receptor gene product exhibiting normal  $\alpha 2M$  receptor gene function, may be inserted into the appropriate

cells within a patient, using vectors that include, but are not limited to adenovirus, adeno-associated virus, and retrovirus vectors, in addition to other particles that introduce DNA into cells, such as liposomes.

Gene replacement therapy techniques should be capable of delivering  $\alpha 2M$  receptor gene sequences to cell types that express the HSP receptor within patients. Thus, in one embodiment, techniques that are well known to those of skill in the art (see, *e.g.*, PCT Publication No. WO89/10134, published April 25, 1988) can be used to enable  $\alpha 2M$  receptor gene sequences to be delivered to developing cells of the myeloid lineage, for example, to the bone marrow. In another specific embodiment, gene replacement can be accomplished using macrophages *in vitro*, and delivered to a patient using the techniques of adoptive immunotherapy.

In another embodiment, techniques for delivery involve direct administration of such  $\alpha 2M$  receptor gene sequences to the site of the cells in which the  $\alpha 2M$  receptor gene sequences are to be expressed, *e.g.*, directly at the site of the tumor.

Additional methods that may be utilized to increase the overall level of  $\alpha 2M$  receptor gene expression and/or  $\alpha 2M$  receptor gene product activity include the introduction of appropriate  $\alpha 2M$  receptor-expressing cells, preferably autologous cells, into a patient at positions and in numbers that are sufficient to ameliorate the symptoms of an  $\alpha 2M$  receptor disorder. Such cells may be either recombinant or non-recombinant.

Among the cells that can be administered to increase the overall level of  $\alpha 2M$  receptor gene expression in a patient are cells that normally express the  $\alpha 2M$  receptor gene.

Alternatively, cells, preferably autologous cells, can be engineered to express  $\alpha 2M$  receptor gene sequences, and may then be introduced into a patient in positions appropriate for the amelioration of the symptoms of an  $\alpha 2M$  receptor disorder or a proliferative or viral disease, *e.g.*, cancer and tumorigenesis. Alternately, cells that express an unimpaired  $\alpha 2M$  receptor gene and that are from a MHC matched individual can be utilized, and may include, for example, brain cells. The expression of the  $\alpha 2M$  receptor gene sequences is controlled by the appropriate gene regulatory sequences to allow such expression in the necessary cell types. Such gene regulatory sequences are well known to the skilled artisan. Such cell-based gene therapy techniques are well known to those skilled in the art, see, *e.g.*, Anderson, U.S. Patent No. 5,399,349.

When the cells to be administered are non-autologous cells, they can be administered using well known techniques that prevent a host immune response against the introduced cells from developing. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does not allow the introduced cells to be recognized by the host immune system.

#### 5.6.4 DELIVERY OF SOLUBLE $\alpha$ 2M RECEPTOR POLYPEPTIDES

Genetically engineered cells that express soluble  $\alpha$ 2M receptor ECDs or fusion proteins, *e.g.*, fusion Ig molecules can be administered *in vivo* where they may function as "bioreactors" that deliver a supply of the soluble molecules. Such soluble  $\alpha$ 2M receptor polypeptides and fusion proteins, when expressed at appropriate concentrations, should neutralize or "mop up" HSPs or other native ligand for the  $\alpha$ 2M receptor, and thus act as inhibitors of  $\alpha$ 2M receptor activity and may therefore be used to treat HSP- $\alpha$ 2M receptor-related disorders and diseases, such as autoimmune disorders, proliferative disorders, and infectious diseases.

10

#### 5.6.5 DELIVERY OF DOMINANT NEGATIVE MUTANTS

In another embodiment of the invention, dominant negative mutants ("dominant negatives") may be used therapeutically to block the immune response to an HSP-antigen complex, *e.g.*, to treat an auto-immune disorder. In general, such dominant-negatives are mutants which, when expressed, interact with ligand (*i.e.*, HSP-antigenic molecule complex), but lack one or more functions, *i.e.* endocytotic functions and/or signaling functions, of normal  $\alpha$ 2MR. Such mutants interfere with the function of normal  $\alpha$ 2MR in the same cell or in a different cell, *e.g.* by titration of HSP-peptide complexes from the wild type receptor. Such a mutation, for example, can be one or more point mutation(s), a deletion, insertion, or other mutation in either the extracellular of the 515 kDa subunit, or the extracellular, transmembrane or intracellular domains of the 85 kDa subunit of the alpha(2) macroglobulin receptor (*see* Krieger and Herz, 1994, *Annu. Rev. Biochem* 63:601-637 for  $\alpha$ 2MR subunit configuration). However, in construction of dominant negative mutations in the either subunit, care should be taken to ensure that the cleavage domain (signaling cleavage between aas 3525 and 3526 of the precursor of  $\alpha$ 2MR) remains intact so that the 515 kDa subunit is processed and presented on the cell surface. Additionally, care should be taken to ensure that the domains by which the two subunits associate should also remain functional. For example, in a specific embodiment, the C-terminal intracellular domain of the 85 kDa subunit is truncated. In another embodiment, a point mutation on the N-terminal 515 kDa subunit blocks endocytosis but not ligand binding. In another embodiment, the N-terminal 515 kDa subunit is expressed as a fusion protein, wherein the C-terminus of said fusion protein is the transmembrane domain and optionally the intracellular domain, of another Type I single transmembrane receptor.

Expression of a such a dominant negative mutation in cell can block uptake of ligand by normal functional receptors in the same or neighboring cells by titrating out the amount of available ligand. Thus, a recombinant antigen presenting cell expressing such a dominant

negative can be used to titrate out HSP-antigenic molecule complexes when administered to a patient in need of treatment for an autoimmune disorder.

#### 5.6.6 EXTRACORPOREAL METHODS FOR MODULATING THE IMMUNE RESPONSE

5 The present invention also relates to methods for modulating an immune response in a patient by altering the levels  $\alpha$ 2M receptor ligand in the bloodstream using extracorporeal methods.  $\alpha$ 2M receptor acts as a heat shock protein receptor in  $\alpha$ 2M receptor-expressing cells, such as macrophages and dendritic cells. Binding of HSPs or HSP antigenic peptide complexes to such  $\alpha$ 2M receptor-expressing cells results in internalization of the HSP and the re-presentation of peptides chaperoned by the HSP. However, because  $\alpha$ 2M receptor has a diverse roles in different cell types and binds numerous non-HSP ligands, competition between  $\alpha$ 2M receptor ligands reduces the ability of HSPs and HSP complexes to access  $\alpha$ 2M receptor.

15 The Applicant has discovered that depleting the blood of non-HSP- $\alpha$ 2M receptor ligands and transfusing such  $\alpha$ 2M receptor-ligand-depleted blood into the bloodstream of a patient can be used to stimulate the immune response, perhaps by increasing access of HSP complexes to the  $\alpha$ 2M receptor. Alternatively, blood can be depleted of  $\alpha$ 2M receptor ligands, including HSPs, followed by the addition of HSPs or HSP antigenic peptide complexes to stimulate a specific immune response. Decreasing the levels of  $\alpha$ 2M receptor ligands can be used to enhance a desired immune response in patients, such as patients with cancer and infectious disease. Such methods for depletion of  $\alpha$ 2M receptor ligands to the bloodstream are described in detail below.

25 In various embodiments, extracorporeal procedures, such as transfusion and apheresis, may be used to stimulate an immune response by modulating  $\alpha$ 2M receptor ligand levels in a patient's circulation or alternatively, depleting  $\alpha$ 2M receptor ligands including HSPs from the blood, followed by the selective addition of specific HSPs or HSP antigenic peptide complexes to the blood. For example, in one embodiment, apheresis techniques coupled with affinity column technology, are used to remove  $\alpha$ 2M receptor ligand from a patient's blood, followed by the return the ligand-depleted blood into circulation.

30 In another embodiment, apheresis techniques coupled with affinity chromatography techniques are used to remove  $\alpha$ 2M receptor ligand from a patient's blood followed by the selective addition of HSPs or HSP antigenic peptide complexes to the patient's blood, and return of the treated blood into the patient's circulation.

35 Extraction of blood can be performed either manually or by any one of the common automated, electronically controlled "apheresis" systems such as the Autopheresis-C.RTM. system (Baxter Healthcare Corporation, Fenwal Division, 1425 Lake Cook Road, Deerfield,

Ill. 60015). In a preferred embodiment, a blood separation apparatus is fluidly connected to a blood vessel of the patient by way of a blood extraction tube. A blood pump, such as a peristaltic pump, is positioned on the blood extraction tube to pump blood from the patient to a blood separation apparatus. An anticoagulant, such as heparin, can be added to the blood through a separate chamber that is in fluid communication with the apheresis system.

Optionally, blood can be taken out of the apheresis system, treated to remove a  $\alpha 2M$  receptor ligand in the laboratory, and then put back into the apheresis system to be reintroduced to the patient. In another embodiment, the blood can be further separated into cellular components such that only a specific subset of cells (*i.e.* leukocytes) can be treated to remove an  $\alpha 2M$  receptor ligand and returned to the patient or, alternatively, only the plasma can be treated to remove an  $\alpha 2M$  receptor ligand and returned to the patient. In another embodiment, after the blood has been treated to remove an  $\alpha 2M$  receptor ligand, HSPs are added back to the blood.

In various embodiments, blood from a patient can be withdrawn manually and the cells can be separated by a standard laboratory blood cell collection device. After or during the cellular collection, the blood can be treated to remove an  $\alpha 2M$  receptor ligand. The cells can then be returned to the patient by an i.v. drip or by injection with a syringe.

In one embodiment, transfusion/apheresis methods may be used to enhance an immune response.  $\alpha 2M$  receptor ligands are removed from transfused blood of a patient in need of treatment for an immune disorder. In another embodiment, the  $\alpha 2M$  receptor ligand that is removed from the blood is not a heat shock protein.

One example of such a method comprises the following steps: (1) withdrawing blood from a patient; (2) passing the patient's blood over an affinity column comprising a  $\alpha 2M$  receptor ligand-binding compound, such as an antibody specific for a  $\alpha 2M$  receptor ligand, for a time period and under conditions sufficient to allow binding of  $\alpha 2M$  receptor ligand to the affinity column; (3) returning the  $\alpha 2M$  receptor-ligand depleted blood to the patient.

In another embodiment, apheresis methods may be used to enhance an immune response by depleting  $\alpha 2M$  receptor ligands (including HSPs) followed by the addition of selective HSPs or HSP antigenic peptide complexes to the blood of a patient.

An example of such a method comprises the following steps: (1) withdrawing blood from a patient; (2) passing the patient's blood over an affinity column comprising a  $\alpha 2M$  receptor-ligand-binding compound for a time period and under conditions sufficient to allow binding of the  $\alpha 2M$  receptor ligand to the affinity column; (3) adding HSPs or HSP antigenic peptide complexes to the ligand depleted blood; (4) returning the blood to the patient.

Methods that can be used to remove a ligand from the blood include affinity chromatography, anion or cation exchange chromatography, phosphocellulose chromatography, immunoaffinity chromatography, hydroxyapatite chromatography, and

lectin chromatography. Affinity purification is based on the interaction between the compound on the affinity column and its binding partner. The principle of affinity chromatography is well known in the art. In one embodiment, a recombinantly expressed and purified (or partially purified) protein, such as  $\alpha 2M$  receptor, is covalently or non-covalently coupled to a solid support such as, for example, a chromatography column. The extracted blood from a patient can be run over such a column. The coupled protein will bind to the  $\alpha 2M$  receptor ligand and deplete the blood of the  $\alpha 2M$  receptor ligand. The depleted blood can then be returned to the patient. In another embodiment, an antibody specific to the ligand can be coupled to the chromatography column and the immunospecific binding of an antibody to the  $\alpha 2M$  receptor ligand can be used to deplete the blood of the  $\alpha 2M$  receptor ligand. Alternatively, one of the many cation or anion exchange resins commonly used in the art can be used to deplete the blood of the  $\alpha 2M$  receptor ligand.

In another embodiment, the present invention also includes a kit that comprises a solid phase chromatography column with a purified  $\alpha 2M$  receptor ligand binding molecule attached thereto. Such a kit can contain components necessary for extracorporeal removal of  $\alpha 2M$  receptor ligands from the blood of a patient in need of such treatment.

Transfusion/apheresis methods may also be used in combination with other methods of immunotherapy. In one embodiment, for example, after depletion of non-HSP  $\alpha 2M$  receptor ligands as described above, HSP-antigenic peptide complexes may be delivered to a cancer patient, or a patient having an infectious disease, using the transfusion/apheresis methods, or other method. Using transfusion/apheresis, at the same time as HSP-antigenic peptide complexes are being delivered,  $\alpha 2M$  receptor ligands (other than HSPs) may be removed from the patient's blood, in order to stimulate the immune response against the HSP-antigenic peptide complex being delivered. Thus, the transfusion/apheresis method makes it possible to accomplish both the delivery of HSP-antigenic peptide complexes and the removal of competing  $\alpha 2M$  receptor ligands in a single procedure.

## 5.7 TARGET AUTOIMMUNE DISEASES

Autoimmune diseases that can be treated by the methods of the present invention include, but are not limited to, insulin dependent diabetes mellitus (*i.e.*, IDDM, or autoimmune diabetes), multiple sclerosis, systemic lupus erythematosus, Sjogren's syndrome, scleroderma, polymyositis, chronic active hepatitis, mixed connective tissue disease, primary biliary cirrhosis, pernicious anemia, autoimmune thyroiditis, idiopathic Addison's disease, vitiligo, gluten-sensitive enteropathy, Graves' disease, myasthenia gravis, autoimmune neutropenia, idiopathic thrombocytopenia purpura, rheumatoid arthritis, cirrhosis, pemphigus vulgaris, autoimmune infertility, Goodpasture's disease, bullous

pemphigoid, discoid lupus, ulcerative colitis, and dense deposit disease. The diseases set forth above, as referred to herein, include those exhibited by animal models for such diseases, such as, for example non-obese diabetic (NOD) mice for IDDM and experimental autoimmune encephalomyelitis (EAE) mice for multiple sclerosis.

5 The methods of the present invention can be used to treat such autoimmune diseases by reducing or eliminating the immune response to the patient's own (self) tissue, or, alternatively, by reducing or eliminating a pre-existing autoimmune response directed at tissues or organs transplanted to replace self tissues or organs damaged by the autoimmune response.

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### 5.8 TARGET INFECTIOUS DISEASES

The infectious diseases that can be treated or prevented using the methods and compositions of the present invention include those caused by intracellular pathogens such as viruses, bacteria, protozoans, and intracellular parasites. Viruses include, but are not limited  
 15 to viral diseases such as those caused by hepatitis type B virus, parvoviruses, such as adeno-associated virus and cytomegalovirus, papovaviruses such as papilloma virus, polyoma viruses, and SV40, adenoviruses, herpes viruses such as herpes simplex type I (HSV-I), herpes simplex type II (HSV-II), and Epstein-Barr virus, poxviruses, such as variola (smallpox) and vaccinia virus, RNA viruses, including but not limited to human  
 20 immunodeficiency virus type I (HIV-I), human immunodeficiency virus type II (HIV-II), human T-cell lymphotropic virus type I (HTLV-I), and human T-cell lymphotropic virus type II (HTLV-II); influenza virus, measles virus, rabies virus, Sendai virus, picornaviruses such as poliomyelitis virus, coxsackieviruses, rhinoviruses, reoviruses, togaviruses such as rubella virus (German measles) and Semliki forest virus, arboviruses, and hepatitis type A  
 25 virus.

In another embodiment, bacterial infections can be treated or prevented such as, but not limited to disorders caused by pathogenic bacteria including, but not limited to, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoea*, *Neisseria meningitidis*, *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Clostridium perfringens*,  
 30 *Clostridium tetani*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, *Klebsiella rhinoscleromatis*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter (Vibrio) fetus*, *Campylobacter jejuni*, *Aeromonas hydrophila*, *Bacillus cereus*, *Edwardsiella tarda*, *Yersinia enterocolitica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*,  
 35 *Salmonella typhiimurium*, *Salmonella typhi*, *Treponema pallidum*, *Treponema pertenuis*, *Treponema carateneum*, *Borrelia vincentii*, *Borrelia burgdorferi*, *Leptospira icterohemorrhagiae*, *Mycobacterium tuberculosis*, *Toxoplasma gondii*, *Pneumocystis carinii*,

*Francisella tularensis*, *Brucella abortus*, *Brucella suis*, *Brucella melitensis*, *Mycoplasma* spp., *Rickettsia prowazeki*, *Rickettsia tsutsugumushi*, *Chlamydia* spp., and *Helicobacter pylori*.

In another preferred embodiment, the methods can be used to treat or prevent  
5 infections caused by pathogenic protozoans such as, but not limited to, *Entamoeba histolytica*, *Trichomonas tenax*, *Trichomonas hominis*, *Trichomonas vaginalis*, *Trypanosoma gambiense*, *Trypanosoma rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, *Leishmania tropica*, *Leishmania braziliensis*, *Pneumocystis pneumonia*, *Plasmodium vivax*, *Plasmodium falciparum*, and *Plasmodium malariae*.

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## 5.9 TARGET PROLIFERATIVE CELL DISORDERS

With respect to specific proliferative and oncogenic disease associated with HSP- $\alpha$ 2M receptor activity, the diseases that can be treated or prevented by the methods of the present invention include, but are not limited to: human sarcomas and carcinomas, e.g.,  
15 fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland  
20 carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilms' tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma,  
25 hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma; leukemias, e.g., acute lymphocytic leukemia and acute myelocytic leukemia (myeloblastic, promyelocytic, myelomonocytic, monocytic and erythroleukemia); chronic leukemia (chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia); and polycythemia vera, lymphoma (Hodgkin's disease and non-  
30 Hodgkin's disease), multiple myeloma, Waldenström's macroglobulinemia, and heavy chain disease.

Diseases and disorders involving a deficiency in cell proliferation or in which cell proliferation is desired for treatment or prevention, and that can be treated or prevented by  
35 inhibiting the  $\alpha$ 2M receptor function, include but are not limited to degenerative disorders, growth deficiencies, hypoproliferative disorders, physical trauma, lesions, and wounds; for

example, to promote wound healing, or to promote regeneration in degenerated, lesioned or injured tissues, etc.

## 5.10 PHARMACEUTICAL PREPARATIONS AND METHODS OF ADMINISTRATION

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The compounds that are determined to affect  $\alpha 2M$  receptor gene expression or gene product activity can be administered to a patient at therapeutically effective doses to treat or ameliorate a cell proliferative disorder. A therapeutically effective dose refers to that amount of the compound sufficient to result in amelioration of symptoms of such a disorder.

10

### 5.10.1 EFFECTIVE DOSE

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the  $LD_{50}$  (the dose lethal to 50% of the population) and the  $ED_{50}$  (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio  $LD_{50}/ED_{50}$ . Compounds that exhibit large therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

20

The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the  $ED_{50}$  with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the  $IC_{50}$  (*i.e.*, the concentration of the test compound that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

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### 5.10.2 FORMULATIONS AND USE

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients.

5 Thus, the compounds and their physiologically acceptable salts and solvates may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically  
10 acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulphate). The tablets may be coated by methods well  
15 known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as  
suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats);  
20 emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-*p*-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled  
25 release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from  
30 pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.*, gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound  
35 and a suitable powder base such as lactose or starch.

The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit

dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for  
5 constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be  
10 formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

15 The compositions may, if desired, be presented in a pack or dispenser device that may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

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## 6. EXAMPLE: IDENTIFICATION OF $\alpha$ 2M RECEPTOR AS AN HSP RECEPTOR

### 6.1 INTRODUCTION

The Example presented herein describes the successful identification of an interaction  
25 between gp96, hsp90, hsp70, and calreticulin with the  $\alpha$ 2M receptor present in macrophages and dendritic cells. The experiments presented herein form the basis for isolating  $\alpha$ 2M receptor polypeptides and for the screening, diagnostic, and therapeutic methods of the present invention.

The Applicant of the present invention noted that certain observations were  
30 inconsistent with a "direct transfer" model of HSP-chaperoned peptide antigen presentation. First, the immunogenicity of HSP preparations is dependent on the presence of functional phagocytic cells but not B cells or other nonprofessional antigen-presenting cells, (Udono and Srivastava, 1993, *supra*; Suto and Srivastava, 1995, *supra*), whereas free peptides can sensitize all cell types. Second, extremely small quantities of HSP-peptide complexes were  
35 effective in eliciting specific immunity, *i.e.*, gp96-chaperoned peptides are several hundred times as effective as free peptides in sensitizing macrophages for CTL recognition,

suggesting the possibility of a specific uptake mechanism. Third, gp96-chaperoned peptides elicited an MHC I response that was not limited by the size of peptide. Finally, the processing of gp96-peptide complexes in macrophage was found to be sensitive to Brefeldin A (BFA), which blocks transport through the Golgi apparatus, suggesting that processing occurred through an intercellular mechanism. These observations led to the hypothesis that HSP-chaperoned peptides may be processed internally and re-presented by MHC class I molecules on the cell surfaces of macrophages (Suto and Srivastava, 1995, *supra*). There is also the hypothesis that the mannose receptor is used in the uptake of gp96 but no mechanism has been proposed for the non-glycosylated HSPs, such as HSP70 (Ciupitu *et al.*, 1998, *J. Exp. Med.*, 187: 685-691). Others suggested that a novel intracellular trafficking pathway may be involved for the transport of peptides from the extracellular medium into the lumen of ER (Day *et al.*, 1997, *Proc. Natl. Acad. Sci.* 94:8065-8069; Nicchitta, 1998, *Curr. Opin. in Immunol.* 10:103-109). Further suggestions include the involvement of phagocytes which (a) possess an ill-defined pathway to shunt protein from the phagosome into the cytosol where it would enter the normal class I pathway; (b) digest ingested material in lysosomes and regurgitate peptides for loading on the surface to class I molecules (Bevan, 1995, *J. Exp. Med.* 192:639-41). The discovery of a receptor for heat shock proteins as disclosed herein helps to resolve the paradox of how extracellular antigenic peptides complexed to HSPs can be presented by MHC class I molecules on antigen presenting cells.

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## 6.2 MATERIALS AND METHODS

*Mice, cells, and reagents.* C57Bl/6, BALB/c and TAP(-/-) mice were obtained from Jackson laboratories. Bone marrow-derived DCs were generated from the femurs and tibia of C57BL/6 mice. The bone marrow was flushed out and the leukocytes obtained and cultured as described (Lutz *et al.*, 1999, *J. Immunol. Methods* 223:77-92) in complete RPMI1640 with 10% heat inactivated FCS and 20ng/ml GMCSF (Endogen Inc., Woburn, MA) for 6 days. On day 3 fresh media with GMCSF was added to the plates for the day 6 cultures. Macrophages were obtained from PEMs of pristaned mice by positive selection for CD11b+ cells (Miltenyi Biotech Inc.). RAW264.7 was gift of Dr. Christopher Nicchitta. A20.25 was gift of Dr. Lawrence Kwak. All other cell lines were obtained from ATCC. Proteasome inhibitor Lactacystin was purchased from Kamiya Inc. Japan. Anti-CD91 antibody (clone 5A6) was purchased from PRAGEN (Heidelberg). Anti-hsp70 (clone N27F3) and anti-PDI (clone 1D3) antibodies were purchased from StressGen (Victoria, Canada).

*Purification of HSPs.* HSPs were purified as described (Srivastava, P.K., 1997, *Methods: A companion to Methods in Enzymology* 12:165-171; Basu and Srivastava, 1999, *J. Exp. Med.* 189(5):797-802). All buffers used for purifications were prepared with endotoxin free water (Nanopure Infinity UV/UF, Barnstead/Thermolyne, Dubuque, IA) and

all glasswares used for purification were cleaned with endotoxin free water and baked in a 4000F oven (Gruenberg, Williamsport, PA). The HSP-containing fractions were identified by immunoblots.

*Conjugation of proteins to FITC and staining of cells.* Purified proteins were  
5 conjugated to FITC using the FluoroTag FITC conjugation kits (SIGMA) as per the  
manufacturers protocol. Conjugation was confirmed by a 2kDa increase in molecular weight  
by SDS-PAGE and by immunoblotting with an anti-FITC monoclonal antibody. Incubations  
of indicated amounts of FITC-tagged proteins and cells were done in the presence of 1%  
nonfat dry milk (Carnation®) in PBS for 20min at 4°C. After repeated washing, cells were  
10 analyzed by flow cytometry (Becton Dickenson, La Jolla, California). Cells were also labeled  
with propidium iodide just before FACScan analysis. Cells staining positive for propidium  
iodide were gated out of the events. No differences were observed in the binding of HSPs to  
Mac-1+ cells from pristaned or non-pristaned mice. Fixed or unfixed cells were labeled with  
FITC-tagged HSP as above. Labeled cells were visualized using a Zeiss LSM confocal  
15 microscope.

*Affinity chromatography.* Proteins (1mg) in 2ml volume were incubated with 2ml of  
equilibrated AminoLink beads in PBS with a reductant (NaCNBH<sub>3</sub>) for 1 hour. Uncoupled  
protein was removed by extensive washing of the column and unreactive groups quenched.  
Immobilization yields were typically >92% of the starting amount of protein. Columns were  
20 stored at 4°C until used. Such columns were made with gp96 (purified as described in  
Srivastava *et al.*, 1986, Proc. Natl. Acad. Sci., U.S.A. 83:3407-3411) and albumin. For  
membrane purification, cells were lysed by dounce homogenization in hypotonic buffer  
containing PMSF. Unlyzed cells and nuclei were removed by centrifugation at 1000g for 5  
mm. The postnuclear supernatant was centrifuged at 100,000g for 90 mins. The pellet  
25 contains total membranes and was fractionated by aqueous two-phase partition with a  
dextran/polyethylene glycol biphasic. Briefly membranes were resuspended in PEG (33%  
wt/wt in 0.22 M sodium phosphate buffer, pH 6.5) and underlaid gently with dextran  
(20%wt/wt in 0.22M sodium phosphate buffer, pH 6.5). The two phases were mixed gently  
and centrifuged at 2000 g for 15 mins. The white material at the interphase was enriched for  
30 plasma membranes, whose proteins were extracted by 2 hr incubation in 20mM Tris buffer  
(pH8, containing 0.08% octylglucoside) at 4°C.

*Photo cross-linking of gp96 to putative receptor.* The cross-linker (SASD, (Pierce)  
was labeled with I<sup>125</sup> using iodobeads (Pierce). Radiolabeled SASD was covalently attached  
to gp96 by incubation at room temperature for 1 hr. Free SASD and I<sup>125</sup> were removed by  
35 size exclusion column (KwikSep columns, Pierce). For cross-linking studies, I<sup>125</sup>-SASD-  
gp96 (50 µg gp96) was incubated with purified CD11b<sup>+</sup> cells. Unbound protein was  
removed by washing. All procedures to this point were carried out in very dim light.

Proteins were cross-linked with UV light. Cells were lysed with lysis buffer (0.5%NP40, 10mM Tris, 1mMEDTA, 150mM NaCl) and treated with 100 mM 2-mercaptoethanol to cleave the cross-linker. *Cell lysates were analyzed by SDS-PAGE and autoradiography.*

*Re-presentation assays.* Re-presentation assays were carried out as described (Suto and Srivastava, 1995, Science 269:1585-1588). Antigen presenting cells (RAW264.7 macrophage cell line) were plated at a 1:1 ratio with AH I -specific T cells in complete RPMI. Approximately 10,000 cells of each type were used. Gp96 (10 µg/ml) chaperoning the AH1-20 mer peptide (RVTYHSPSYVYHQFERRAK) was added to the cells and the entire culture was incubated for 20 hrs. Stimulation of T cells was measured by quantifying the amount of IFN-γ released into the supernatants by ELISA (Endogen). In addition, CD11b+ peritoneal exudate cells (1X104) were pulsed with HSPs purified from liver, or HSP-peptide complex generated in vitro and relevant CD8+ T cells (VSV8 specific CTL line or AH1-specific CTL clones, as indicated) were added to the cultures. The assay was carried out in 250 ml volume in 96-well plates with RPMI medium containing 5% FCS at 37°C for 20 hours. Culture supernatants were harvested and tested for the presence of IFN-γ release by ELISA (Endogen Inc., Woburn, MA).

*Complexing in vitro of peptide to HSPs.* HSPs were mixed with VSV19 or AH1-19 in a 50 : 1 peptide to protein molar ratio in 0.7M NaCl in Na - phosphate buffer and heated at 500 C for 10 min., then incubated at room temperature for 30 min. Excess free peptide was removed with PBS using centricon 10 ( Amicon, Inc., Beverly MA).

*Purification of CD11b+ cells.* CD11b+ cells were selected using the MACS columns and protocols supplied by Miltenyi Biotec Inc. Auburn, California. CD11b antibody, supplied as CD11b MicroBeads, was purchased from Miltenyi Biotec Inc., and has been demonstrated not to activate CD11b+ cells with regard to the markers tested in this experiment.

*Induction of cytotoxic T cells.* C57BL/6 mice were immunized intraperitoneally with 50 mg of gp96 complexed with VSV19 peptide. Ten days later, recipient spleens were removed and splenocytes were stimulated with VSV8 synthetic peptide at 1mM concentration. After 5 days, MLTCs were tested for cytotoxicity in a chromium release assay using EL4 cells alone and EL4 cells pulsed with VSV8 peptide as targets.

*Protein Microsequencing.* Proteins identified by affinity chromatography were analyzed on SDS-PAGE and stained with coomassie blue or transferred onto PVDF membrane and stained with coomassie blue, all of it under keratin-free conditions. Protein bands were excised with a razor from the gel or membrane. Tryptic peptides from an 80kDa faint coomassie band were extracted by 50% acetonitrile, 5% formic acid, dried, and loaded onto a 75 m 10 cm, reverse-phase C18, microcapillary column (3 µl vol) and tryptic peptides were separated by on-line microcapillary liquid chromatography-tandem mass spectrometry

followed by database searching using the SEQUEST program as previously described. (Gatlin *et al.*, 2000, *Anal. Chem.* 72:757-63; Link *et al.*, 1999, *Nat. Biotechnol.* 17:676-82). The analysis was carried out in a data-dependent auto-MS/MS fashion using a Finnigan LCQ iontrap Mass Spectrometer.

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### 6.3 RESULTS

*Identification of an 80 kDa protein as a potential gp96 receptor.* Homogenous preparations of gp96 were coupled to FITC and the gp96-FITC was used to stain RAW264.7 cells, shown to be functionally capable of re-presenting gp96-chaperoned peptides. Gp96-FITC but not control albumin-FITC preparations stained the cell surface of RAW264.7 cells (FIG. 1A). Plasma membrane preparations of cell surface-biotinylated RAW264.7 cells were solubilized in 0.08% octyl-glucoside and the soluble extract was applied to a gp96-Sepharose column. The bound proteins were eluted with 3M sodium chloride. SDS-PAGE analysis of the eluate showed 2 major bands of ~75-80 kDa size (FIG. 1B, top left). Blotting of this gel with avidin-peroxidase showed that both bands were biotinylated, indicating their surface localization (FIG. 1B, bottom left). Affinity purification of membrane extracts of RAW264.7 cells over control serum albumin affinity columns did not result in isolation of any proteins, nor did probing of immunoblots of such gels with avidin peroxidase detect any albumin-binding surface proteins (FIG. 1B, top and bottom center lanes). As an additional control, chromatography of membrane extracts of P815 cells which do not bind gp96-FITC and which do not re-present gp96-chaperoned peptides, on gp96 affinity columns did not result in elution of any gp96-binding proteins (FIG. 1B, top and bottom right lanes).

In parallel experiments, gp96 molecules were coupled to the radio-iodinated linker sulfosuccinimidyl (4-azidosalicylamido) hexanoate (SASD) which contains a photo cross-linkable group. Gp96-SASD- $I^{125}$  was pulsed onto peritoneal macrophages, which have been shown previously to re-present gp96-chaperoned peptides (Suto and Srivastava, 1995, *Science* 269:1585-1588). Excess gp96-SASD was removed by multiple rounds of washing of the cells and photoactivation was carried out by exposure of cells to UV light for 10 min. Cell lysates were reduced in order to transfer the  $I^{125}$  group to the putative gp96 ligand and were analyzed by SDS- PAGE followed by autoradiography. The gp96 molecule was observed to cross-link to an ~80 kDa band specifically present in re-presentation-competent macrophage but not in the re-presentation-incompetent P815 cells (FIG. 1C). This band appears to correspond in size to the larger of the two bands seen in eluates of gp96 affinity columns (FIG. 1B). No band corresponding to the lower band in that preparation is seen in the photo cross-linked preparation. The observation of a specific binding of gp96 to an 80 kDa protein in two different re-presentation-competent cell types, but not in a re-

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presentation-incompetent cell line, and by two independent assays supported the candidacy of the 80 kDa molecule for the gp96 receptor.

*Antiserum against the 80 kDa protein inhibits re-presentation of a gp96-chaperoned antigenic peptide.* The eluates containing the 75-80 kDa proteins were used to immunize a New Zealand white rabbit, and pre-immune and immune sera were used to probe blots of plasma membrane extracts of the re-presentation-competent RAW264.7 and primary peritoneal macrophages and the re-presentation-incompetent P815 cells. The immune but not the pre-immune serum detected the 80 kDa band (and a faint lower 75 kDa band) in plasma membrane extracts of primary macrophage and the RAW264.7 membranes but not of P815 cells (FIG. 2A). The pre-immune and immune sera were tested in a functional assay for their ability to block re-presentation of gp96-chaperoned peptides. The L<sup>d</sup>-restricted epitope AH1 derived from the gp70 antigen of murine colon carcinoma CT26 (Huang *et al.*, 1996, Proc. Natl. Acad. Sci. U.S.A. 93:9730-9735) was used as the model system. Complexes of gp96 with an AH1 precursor (used to inhibit direct presentation) were pulsed onto RAW264.7 cells which were used to stimulate a L<sup>d</sup>/AH1-specific CD8<sup>+</sup> T cell clone. Release of interferon- $\gamma$  by the clones was measured as a marker of their activation. RAW264.7 cells were able to re-present gp96-chaperoned AH1 precursor effectively in this assay. It was observed that at the highest concentration, the immune sera inhibited re-presentation completely (FIG. 2B). Although the pre-immune serum was ineffective in inhibiting re-presentation as compared to the immune sera, it did inhibit re-presentation significantly at higher concentrations. The significance of this observation became clear later when we determined the identity of the gp96 receptor. Repeated immunizations with the affinity-purified gp96-binding proteins did not result in corresponding increase in antibody titers.

*Identification of the 80 kDa protein as an amino terminal fragment of the heavy chain of the  $\alpha$ 2M receptor.* The 80 kDa protein eluted from the gp96 affinity column was resolved on SDS-PAGE and visualized by staining with Coomassie Brilliant Blue. The protein band was subjected to in-gel trypsin digestion and mass spectrometry-based protein microsequencing as described in the methods in Section 6.2. Four independent tryptic peptides corresponding to N-terminal region of the  $\alpha$  2-macroglobulin ( $\alpha$ 2M) receptor, designated by immunologists as CD91, were identified (FIG. 3C).

*$\alpha$ 2M inhibits re-presentation of a gp96-chaperoned antigenic peptide by RAW264.7.*  $\alpha$ 2M receptor is one of the known natural ligands for the  $\alpha$ 2M receptor. Its ability to inhibit re-presentation of gp96-chaperoned antigenic peptide AH1 was tested in the assay described in FIG. 2.  $\alpha$ 2M but not control proteins selectin (CD62) or serum albumin was observed to inhibit re-presentation completely and titratably (FIG. 4). This observation was also consistent with the result in FIG. 2 that while the pre-immune serum did not detect an 80 kDa band in plasma membranes of RAW264.7 cells, it did inhibit re-presentation to some

degree at high concentrations. Thus, by structural as well as functional criteria, the  $\alpha 2M$  receptor was determined to fulfill the criteria essential for a receptor for gp96.

*Binding of fluorescence-labeled HSPs and  $\alpha_2$ -macroglobulin to a panel of primary and cultured cells.* FITC-labeled HSPs, gp96, hsp90 or hsp70, or control non-HSP serum albumin (SA) were incubated with primary cells such as pristane-induced peritoneal macrophage, differentiated bone marrow-derived dendritic cells or with immortalized cell lines such as RAW264.7, RAW309Cr.1 of macrophage origin, P815 mastocytoma, YAC-1 lymphoma, EL4 thymoma, Meth A and PS-C3H fibrosarcomas, B16 melanoma, CT26 colon carcinoma, and UV6139 squamous cell carcinoma, as described in the Methods. After removal of unbound protein by extensive washing, cells were analyzed by flow cytometry. As shown in Figure 5, the peritoneal macrophages and the bone marrow-derived dendritic cells showed robust binding of each of the three HSPs but not albumin. However, of the two macrophage cell lines, only one of them, RAW264.7, bound the three HSPs.

RAW309Cr.1 did not bind any of the HSPs (FIG. 6A and 6B). Out of 8 other cell lines tested with the FITC-labeled gp96, hsp90 and hsp70, none was observed to bind to HSP in a manner comparable to the binding observed with RAW264.7. YAC 1 was observed to bind hsp70 but only to a significantly smaller degree. The binding was only a fraction of that observed with APCs.

As described above, the  $\alpha 2$  macroglobulin receptor has been identified as the receptor for gp96. All of the cell types in Figure 5 were also tested for the presence of CD91 by staining with FITC- $\alpha 2$  macroglobulin. CD91 showed precisely the same pattern of distribution as did each of the three HSPs (FIG. 5).

*The ability of cells to bind HSPs and  $\alpha_2M$  correlates with the ability to re-present gp96-chaperoned peptides.* We tested if the ability of a particular cell type to bind HSPs or  $\alpha_2$  macroglobulin as shown in Figure 5 correlates with its ability to re-present gp96-chaperoned peptides. Re-presentation studies are done typically by incubating APCs and an HSP, chaperoning a known peptide, with T cells specific for an epitope present in the chaperoned peptide (Suto and Srivastava, 1995, *supra*). The experimental system is set up such that the peptide cannot charge directly onto MHC I but requires intracellular processing followed by presentation to T cells. VSV8 and AH1 antigenic systems were used in these studies. The VSV8 epitope (RGYVYQGL) is presented by the  $K^b$  molecule and VSV19 (SLSDL RGYVYQGLKSGNVS) is its extended variant, which cannot charge  $K^b$  directly. AH1 (SPSYVYHQF) is an  $L^d$ -restricted epitope of a murine leukaemia virus envelope protein gp70 (Huang et al., 1996), and AH1-19 (RVTYHSPSYVYHQFERRAK) is its extended version. Peritoneal macrophage and BM-DCs were tested side-by-side for re-presentation in the VSV8 system, and both cell types were able to re-present gp96-

chaperoned VSV19 to VSV8-specific T cells (FIG. 7A). EL4 and B16 cells, both of the *b* haplotype, were also tested and were found unable to re-present in identical assays (data not shown). The BM-DCs were observed to re-present gp96-chaperoned VSV19 significantly better than macrophage did; however, it is not possible to determine from the data if this difference derives from the better T cell stimulatory properties of DCs in general or whether the DCs are specifically more efficient than macrophage at re-presenting gp96-chaperoned peptides. The two macrophage cell lines RAW309Cr.1 and RAW264.7 were tested for their re-presentation ability in the AH1 system. In parallel with the HSP and  $\alpha$ 2M-staining data (FIG. 5), RAW264.7 cells but not RAW309Cr.1 were observed to be capable of re-presenting gp96-chaperoned AH1 peptides (Fig. 7B).

*Peptides chaperoned by hsp90, hsp70 and CRT are re-presented by MHC I molecules of APCs.* Gp96 was the first HSP for which the re-presentation phenomenon was experimentally shown (Suto and Srivastava 1995, *supra*). Hsp70-chaperoned peptides have been shown recently to be re-presented by APCs (Castellino *et al.*, 2000, J.Exp Med. 191(11):1957-1964). The ability of other HSPs, hsp90 and CRT to introduce chaperoned peptides into the endogenous presentation pathway was tested in the AH1 system with RAW264.7 cells as the APCs. RAW264.7 cells were pulsed with hsp90, hsp70, calreticulin, or gp96, as a positive control, by themselves, or chaperoning the AH1-19 peptide. Chaperoning of peptides by the HSPs was accomplished in vitro as previously described (Blachere *et al.* 1997, J.Exp. Med. 186:1315-1322; Basu and Srivastava 1999, J. Exp. Med.189:797-802). T cells specific for L<sup>d</sup>/AH-1 secreted IFN- $\gamma$  when the RAW264.7 cells were pulsed with complexes of hsp90, hsp70, CRT or gp96 with AH1-19, but not when the HSPs were not complexed with the peptide (FIG. 8). Pulsing of RAW264.7 cells with AH1-19 alone did not lead to surface loading of L<sup>d</sup> molecules and consequent stimulation of T cells. Further, RAW264.7 cells pulsed with complexes of serum albumin with AH1-19, also failed to stimulate L<sup>d</sup>/AH1-specific T cells, thus indicating the specific requirement of HSPs for introducing the chaperoned peptides into the endogenous presentation pathway (FIG. 8).

*Gp96, hsp90, hsp70 and CRT engage a common receptor.* Does each HSP have a unique receptor or do they share a common receptor? This question was addressed by three independent criteria : by measuring re-presentation of gp96-chaperoned AH1-19 (as in FIGS. 7 and 8) in the presence of excess and titrated quantities of free (i.e. not complexed to AH1-19) gp96, hsp90, hsp70 or serum albumin, by testing if  $\alpha$ <sub>2</sub> macroglobulin, a known ligand for CD91, a receptor for gp96, can inhibit re-presentation of peptides chaperoned by gp96, hsp90, hsp70 or CRT, and finally, if anti-CD91 antibody can inhibit re-presentation of peptides chaperoned by some or all the HSPs.

The gp96-AH1-19 complex was added to RAW264.7 cultures at a fixed final concentration of 40  $\mu\text{g/ml}$ , while the competing HSPs or serum albumin were added at concentrations between (200-800)  $\mu\text{g/ml}$ . It was observed (FIG. 9A) that all 3 competing HSPs could inhibit re-presentation of gp96-chaperoned AH1-19, albeit with different efficiencies. Gp96 was able to compete with itself, while hsp90 was an even better competitor than gp96. Hsp70 was a less efficient competitor than gp96 but was a significant competitor. Albumin competed inefficiently. In quantitative terms, approximately 2 fold molar excess of hsp90, 6 fold molar excess of gp96, and a 13 fold molar excess of hsp70 were required to inhibit by 50% the re-presentation of gp96-chaperoned peptides at a gp96 concentration of 40  $\mu\text{g/ml}$ . All three HSPs were able to inhibit the re-presentation of gp96-chaperoned peptides completely at the highest concentration tested. This observation suggests that gp96, hsp90 and hsp70 utilize a single receptor albeit with differing specificities.

In additional experiments, increasing quantities of  $\alpha_2$  macroglobulin were added to re-presentation assays where AH1-19 chaperoned by gp96, hsp90, hsp70 or CRT was re-presented by RAW264.7 cells, to L<sup>d</sup>/AH-1 specific T cells.  $\alpha_2$  macroglobulin was observed to inhibit, in a titratable manner, re-presentation of peptides chaperoned by each of the four HSPs (FIG. 9B). Re-presentation of peptides chaperoned by gp96, hsp70 and CRT was inhibited equally, while re-presentation of hsp90-chaperoned peptide was inhibited the most effectively, and almost completely at the highest concentration of  $\alpha_2$  macroglobulin tested. Serum albumin, when tested at the highest concentration, inhibited re-presentation only modestly. It may be noted that while the data in Fig. 9A show that the specific peptide-deficient HSPs inhibited re-presentation of gp96-AH1-19 complexes completely at the highest concentrations tested,  $\alpha_2$  macroglobulin appears far less effective, in quantitative terms, at inhibiting the re-presentation of peptides chaperoned by 3 of the 4 HSPs (FIG. 9B). However, this quantitative disparity disappears if one notes that the  $\alpha_2$  macroglobulin molecule is approximately 10 times larger in molecular mass than the average HSP molecule.

A mouse monoclonal anti-CD91 IgG<sub>1</sub> antibody and isotype control antibodies were tested for their ability to inhibit re-presentation of peptides chaperoned by gp96, hsp90, hsp70 and CRT. As before, the RAW264.7/AH1 system was utilized and the antibodies were added to re-presentation cultures at the concentrations indicated (Fig. 9C). Anti-CD91 antibody was observed to inhibit, titratably and specifically, the re-presentation of AH1 chaperoned by each of the 4 HSPs tested. The isotype control antibody did not inhibit re-presentation in any instance. Further, the inhibition mediated by the anti-CD91 antibody was complete and uniform for each of the HSPs, indicating that CD91 is the sole receptor for each of the 4 HSPs.

*Requirement of a functional proteasome complex for the representation of gp96-chaperoned peptides by APCs.* The re-presentation assay was carried out in presence or absence of the specific proteasome inhibitor, lactacystin. The peritoneal macrophages were treated or untreated with lactacystin for 2 hr and then cultured with gp96-VSV19 complex for another 2 hr in presence or absence of the inhibitor. The cells were chromium labeled at the same time for 1 hr and then washed and used as targets against CD8<sup>+</sup>T cells specific for VSV8 in a 4 hr chromium release assay. Gp96-VSV19, lactacystin-untreated pulsed APCs were lysed by VSV8-specific CD8<sup>+</sup> T cells (FIG. 10A). As observed previously for gp96 (Suto and Srivastava 1995, *supra*) and for hsp70 (Castellino *et al.*, 2000, *supra*), only a small proportion of pulsed APCs were lysed by the APCs even at the highest E:T ratio tested (FIG. 10A). The APCs pulsed with VSV8 (through surface charging) were lysed in a titratable and more significant degree, indicating that the APCs were entirely capable of being lysed. The basis of the selective lyzability of APCs re-presenting HSP-chaperoned peptides is still unclear. However, and regardless of this observation, the lactacystin-treated, gp96-VSV19 pulsed APCs were not recognized by the VSV8-specific CD8<sup>+</sup> T cells and were not lysed by them (FIG. 10A). Inhibition of proteasomal function thus inhibits the processing of peptides chaperoned by gp96 (FIG.10A). As other HSPs tested also use the same portal of entry (FIG. 9), it is assumed that inhibition of proteasome function interferes with processing of peptides chaperoned by them as well. The data recently reported by Castellino *et al.* for hsp70 are consistent with this inference.

*Re-presentation of gp96-chaperoned peptides by MHC I of the APCs requires a functional TAP.* The requirement of TAP in re-presentation of gp96 chaperoned peptides by APCs was tested. In a re-presentation assay *in vitro*, gp96 purified from liver or the same gp96 complexed with VSV19 was pulsed on to primary cultures of peritoneal macrophages derived from TAP +/+ or -/- mice. The pulsed APCs were used to stimulate CD8<sup>+</sup> T cell lines specific for K<sup>b</sup>/VSV8. After incubation for 20 hr, the culture supernatants were tested for release of IFN- $\gamma$  as a marker for T cell stimulation (FIG.10B). It was observed that APCs from TAP+/+ mice stimulated the CD8<sup>+</sup> T cells specifically when cultured in presence of gp96 complexed to VSV19 but APCs from TAP1-/- mice were unable to do so. This result indicates that gp96-chaperoned peptides must enter the endoplasmic reticulum through the TAP molecules, for being loaded on the MHC I molecules. As other HSPs tested also use the same portal of entry (FIG. 9), it is assumed that peptides chaperoned by other HSPs also require TAP for re-presentation. Part of the data recently reported by Castellino *et al.* for hsp70 are consistent with this inference.

In studies *in vivo*, TAP1(-/-) (C57BL/6/SV129J) or wild type (C57BL/6) mice were immunized with the gp96-VSV19 complexes (50  $\mu$ g of gp96 complexed with 50  $\mu$ g of

VSV19), or VSV19 alone, or gp96 alone. Spleen cells of immunized mice were cultured with the VSV8 and tested for cytotoxic activity on <sup>51</sup>Cr labeled EL4 cells or EL4 cells pulsed with the VSV8 peptide as targets. Spleen cells of wild type (C57BL/6) mice immunized with gp96-peptide complex showed VSV8-specific CTL activity whereas splenocytes of TAP1 (-/-) mice immunized with gp96-peptide complex showed no cytotoxic activity (FIG.10C). It may be argued that the lack of CTL activity in TAP-/- mice is a result of the poor loading and stability of MHC I molecules in general, rather than because of a specific block in re-presentation. While this is possible, and is difficult to entirely refute, we are easily able to generate VSV8-specific CTLs in TAP-/- mice as in TAP+/+ mice by immunization with VSV8 peptide in incomplete Freund's adjuvant (data not shown). Sandberg et al. (1996) have reported similar data. In any case, the data from re-presentation assays in vitro using APCs from TAP+/+ and -/- mice (FIG. 10B) demonstrate the TAP requirement for re-presentation convincingly and without the complexity introduced by the experiment in vivo (FIG. 10C).

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#### 6.4 DISCUSSION

The  $\alpha$ 2M receptor, which is also designated CD91, was initially identified as a protein related to the low density lipoprotein (LDL) receptor Related Protein (LRP) (Strickland *et al.*, 1990, J. Biol. Chem.265:17401-17404; Kristensen *et al.*, 1990, FEBS Lett. 276:151-155). The protein consists of an ~420 kDa  $\alpha$  subunit, an 85 kDa  $\beta$  subunit and a 39 kDa tightly associated molecule (RAP). The  $\alpha$  and  $\beta$  subunits are encoded by a single transcript of ~15 Kb in size (Van Leuven *et al.*, 1993, Biochim. Biophys. Acta. 1173:71-74). The receptor has been shown to be present in cells of the monocytic lineage and in hepatocytes, fibroblasts and keratinocytes. CD91 has been shown previously to bind the activated form of the plasma glycoprotein  $\alpha$ 2M, which binds to and inhibits a wide variety of endoproteinases.  $\alpha$ 2M receptor also binds to other ligands such as transforming growth factor  $\beta$  (O Connor-McCourt *et al.*, 1987, J. Biol. Chem. 262:14090-14099), platelet-derived growth factor (Huang *et al.*, 1984, Proc. Natl. Acad. Sci. U.S.A. 81:342-346), and fibroblast growth factor (Dennis *et al.*, 1989, J. Biol. Chem. 264:7210-7216).  $\alpha$ 2M is thus believed to regulate, and specifically diminish, the activities of its various ligands. Complexed with these various ligands,  $\alpha$ 2M binds  $\alpha$ 2M receptor on the cell surface and is internalized through receptor-mediated endocytosis. Uptake of  $\alpha$ 2M-complexed ligands has been assumed thus far to be the primary function of the  $\alpha$ 2M receptor, although a role for it in lipid metabolism is also assumed.  $\alpha$ 2M receptor ligands other than  $\alpha$ 2M, such as tissue-specific plasminogen activator-inhibitor complex (Orth *et al.*, 1992, Proc. Natl. Acad. Sci. U.S.A. 89:7422-7426) and urokinase-PAI1 complex (Nykjaer *et al.*, 1992, J. Biol. Chem. 267:14543-14546), have been identified. These ligands attest to a role for  $\alpha$ 2M receptor in clearing a range of

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extracellular, plasma products.

The studies reported here show that the heat shock proteins gp96, hsp90, hsp70, and calreticulin are additional ligands for the  $\alpha$ 2M receptor. The human gp96-coding gene has been mapped previously by us at chromosome 12 (q24.2→q24.3) (Maki *et al.*, 1993, Somatic Cell Mol. Gen. 19:73-81). It is of interest in this regard that the  $\alpha$ 2M receptor gene has been mapped to the same chromosome and at a not too distant location (q13→q14) (Hilliker *et al.* Genomics 13:472-474). Gp96 binds  $\alpha$ 2M receptor directly and not through other ligands such as  $\alpha$ 2M. Homogenous preparations of gp96, in solution, or cross-linked to a solid matrix, bind to the  $\alpha$ 2M receptor. Indeed, the major ligand for the  $\alpha$ 2M receptor,  $\alpha$ 2M, actually inhibits interaction of gp96 with  $\alpha$ 2M receptor, instead of promoting it, providing evidence that gp96 is a direct ligand for the  $\alpha$ 2M receptor. The 80 kDa protein shown to bind gp96 is clearly an amino terminal degradation product of the  $\alpha$  subunit of the  $\alpha$ 2M receptor. Degradation products of the  $\alpha$ 2M receptor in this size range have also been observed in previous studies (Jensen *et al.*, 1989, Biochem. Arch. 5:171-176), and may indicate the existence of a discrete ectodomain in the  $\alpha$ 2M receptor which may be particularly sensitive to proteolytic cleavage.

The studies shown here also indicate that the  $\alpha$ 2M receptor is also engaged by hsp90, hsp70 and calreticulin. This observation is surprising in light of the fact that hsp70, calreticulin and hsp90/gp96 have no obvious structural similarities with each other. In another context, HSPs have presented us with this dilemma before: many of the various HSPs have no obvious homologies with each other and yet they appear to bind many of the same peptides (Ishii *et al.*, 1999, J. Immunol. 162(3):1303-1309; Breloer *et al.*, 1998, Eur. J. Immunol. 28(3):1016-1021). It remains to be seen if grp170, which belongs to the extended hsp70 family and hsp110, which has no homology to any of the other HSPs, shall share the CD91 receptor. The multiple common properties of the HSPs which share the Fourth Paradigm (Srivastava P.K., 1994, Experientia 50(11-12):1054-1060), i.e. peptide-binding, interacting with APCs through a common receptor, ATP-binding and ATPase activity, strongly suggest that these molecules must share conformational similarities which are not obvious from their primary sequence. Crystallographic analyses of the HSPs have begun to reveal their structure (Zhu *et al.*, 1996, Science 272:1602-1614; Prodromou *et al.*, 1997, Cell 90:65-75; Stebbins *et al.*, 1997, Cell 89:239-250), and shall shed light on this question.

The observations that  $\alpha$ 2 macroglobulin and anti-CD91 antibodies inhibit re-presentation by each of the four HSPs completely, indicate that CD91 is the only receptor for the 4 HSPs. Considering the increasingly obvious role which the HSPs play in innate (Basu *et al.*, 2000, Int. Immunol. 12(11):1539-1546) and adaptive immune response, this observation is somewhat counter-intuitive. However, the data on complete inhibition by two independent means (FIG.. 9) are quite compelling. We have noticed earlier, and we report

here, significant differences between hsp70 and hsp90/gp96 in their ability to compete for binding to gp96 receptors (Binder *et al.*, 2000, J. Immunol. 165:2582-2587). Another group has also observed similar differences between gp96 and hsp70 (Arnold-Schild *et al.*, 1999, 162:3757-3760). These differences are not inconsistent with our present report pointing to a single receptor for the 4 HSPs. They simply suggest that the various HSPs interact with a single receptor with widely differing affinities. Castellino *et al.* have recently demonstrated re-presentation of hsp70-chaperoned peptides by APCs through receptor-mediated uptake and have suggested the existence of receptors of different affinity classes for single HSPs. This argument is biologically cogent, but is not supported by our present data.

Once the HSP-peptide complex binds to the receptor, peptides chaperoned by the HSPs must enter the APC along with the HSP. The studies reported here address the downstream events solely with respect to gp96 in the assumption that if all HSPs enter through the same portal, the downstream events must be identical or similar for peptides chaperoned by each of them. Our observations suggest that the peptides go from the endosome to the cytosol, to the ER and then to the secretory pathway to be re-presented on the surface. The transit through the cytosol is established through the proteasome requirement as well as through the TAP requirement of re-presentation. There is no known mechanism for transit of molecules from vesicular to soluble compartment although precedents certainly exist (Chiang *et al.*, 1989, Science 246:382-385). Exploration of this pathway shall, without doubt, open a new window into our understanding of intracellular traffic of proteins. Castellino *et al.* have reported recently on the events as they occur downstream of receptor-mediated uptake of hsp70-peptide complexes by APCs (Castellino *et al.*, 2000, *supra*). Our observations with a different HSP (gp96) are entirely consistent with that version of events and buttress the notion that the same portal of entry is used by all the peptide-chaperoning HSPs for re-presentation.

As shown here, the heat shock protein- $\alpha$ 2M receptor interaction provides a new type of function for  $\alpha$ 2M receptor, a function of a sensor, not only of the extracellular environment with its previously known plasma-based ligands, but also a sensor of the intracellular milieu as well. HSPs such as gp96 are obligate intracellular molecules and are released into the extracellular milieu only under conditions of necrotic (but not apoptotic) cell death. Thus, the  $\alpha$ 2M receptor may act as a sensor for necrotic cell death (see FIG. 11), just as the scavenger receptor CD36 and the recently identified phosphatidyl serine-binding protein act as sensors of apoptotic cell death and receptors for apoptotic cells (Savill *et al.*, 1992, J. Clin. Invest.90:1513-1522; Fadok *et al.*, 2000, Nature 405:85-90). Interaction of the macrophages with the apoptotic cells leads to a down-regulation of the inflammatory cytokines such as TNF (Fadok *et al.*, 2000, *supra*), while gp96-APC interaction leads to re-presentation of gp96-chaperoned peptides by MHC I molecules of the APC, followed by

stimulation of antigen-specific T cells (Suto and Srivastava, 1995, *supra*) and, in addition, secretion of pro-inflammatory cytokines such as TNF, GM-CSF and IL-12. Interestingly,  $\alpha 2M$ , an independent ligand for the  $\alpha 2M$  receptor, inhibits representation of gp96-chaperoned peptides by macrophages. This observation suggests that re-presentation of gp96-chaperoned peptides can not occur physiologically in blood, but only within tissues as a result of localized necrotic cell death. This is consistent with the complete absence of gp96 or other HSPs in blood under all conditions tested. Together, these observations point towards a possible mechanism whereby the release of HSPs in the blood as a result of severe tissue injury and lysis will not lead to a systemic and lethal pro-inflammatory cytokine cascade.

It is possible, therefore, that the  $\alpha 2M$  receptor renders it possible for the APCs to sample (i) the extracellular milieu of the blood through  $\alpha 2M$  and other plasma ligands and (ii) the intracellular milieu of the tissues through HSPs, particularly of the gp96 family. The former permits APCs to implement their primordial phagocytic function, while the latter allows them to execute its innate and adaptive immunological functions. Viewed in another perspective, recognition of apoptotic cells by APCs through CD36 or phosphatidyl serine, leads to anti-inflammatory signals, while interaction of the APC with necrotic cells through  $\alpha 2M$  receptor leads to pro-inflammatory innate and adaptive immune responses (see Srivastava *et al.*, 1998, *Immunity* 8: 657-665).

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The invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of the invention. Indeed various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

All references cited herein, including patent applications, patents, and other publications, are incorporated by reference herein in their entireties for all purposes.

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**WHAT IS CLAIMED IS:**

1. A method for identifying a compound that modulates an HSP- $\alpha$ 2M receptor-mediated process, comprising:
  - 5 (a) contacting a test compound with a heat shock protein and an alpha (2) macroglobulin receptor; and
  - (b) measuring the level of alpha (2) macroglobulin receptor activity or expression,such that if the level of activity or expression measured in (b) differs from the level of alpha  
10 (2) macroglobulin receptor activity in the absence of the test compound, then a compound that modulates an HSP- $\alpha$ 2M receptor-mediated process is identified.
2. The method of Claim 1, in which the compound identified is an antagonist which interferes with the interaction of the heat shock protein with the alpha (2)  
15 macroglobulin receptor, further comprising the step of:
  - (c) determining whether the level interferes with the interaction of the heat shock protein and the alpha (2) macroglobulin receptor.
3. The method of Claim 1, in which the test compound is an antibody specific  
20 for the alpha (2) macroglobulin receptor.
4. The method of Claim 1, in which the test compound is an antibody is specific for alpha (2) macroglobulin.
- 25 5. The method of Claim 1, in which the test compound is an antibody is specific for a heat shock protein.
6. The method of Claim 1, in which the test compound is a small molecule.
- 30 7. The method of Claim 1, in which the test compound is a peptide.
8. The method of Claim 7, in which the peptide comprises at least 5 consecutive amino acids of the alpha (2) macroglobulin receptor (SEQ ID NO.: 7).
- 35 9. The method of Claim 7, in which the peptide comprises at least 5 consecutive amino acids of alpha (2) macroglobulin (SEQ ID NO.: 4).

10. The method of Claim 7, in which the peptide comprises at least 5 consecutive amino acids of a heat shock protein sequence.

11. The method of Claim 1, in which the compound is an agonist which enhances  
5 the interaction of the heat shock protein with the alpha (2) macroglobulin receptor.

12. The method of Claim 1 in which the HSP- $\alpha$ 2M receptor-mediated process affects an autoimmune disorder, a disease or disorder involving disruption of antigen presentation or endocytosis, a disease or disorder involving cytokine clearance or  
10 inflammation, a proliferative disorder, a viral disorder or other infectious disease, hypercholesterolemia, Alzheimer's disease, diabetes, or osteoporosis.

13. A method for identifying a compound that modulates an HSP- $\alpha$ 2M receptor-mediated process, comprising:

- 15 (a) contacting a test compound with a heat shock protein and an alpha (2) macroglobulin receptor-expressing cell; and  
(b) measuring the level of alpha (2) macroglobulin receptor activity or expression in the cell,

such that if the level of activity or expression measured in (b) differs from the level of alpha  
20 (2) macroglobulin receptor activity in the absence of the test compound, then a compound that modulates an HSP- $\alpha$ 2M receptor-mediated process is identified.

14. The method of Claim 1 or 13 wherein the alpha (2) macroglobulin receptor activity measured is the ability to interact with a heat shock protein.

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15. The method of Claim 13 wherein the heat shock protein is non-covalently associated with an antigenic peptide and the alpha (2) macroglobulin receptor activity measured is the ability to re-present the antigenic peptide.

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16. The method of Claim 13 wherein the heat shock protein is non-covalently associated with an antigenic peptide and the alpha (2) macroglobulin receptor activity measured is the ability to stimulate a cytotoxic T cell response against the antigenic peptide.

17. A method for identifying a compound that modulates the binding of a heat  
35 shock protein to the  $\alpha$ 2M receptor, comprising:

- (a) contacting a heat shock protein with an alpha (2) macroglobulin receptor, or fragment, or analog, derivative or mimetic thereof, in the presence of a test

compound; and

- (b) measuring the amount of heat shock protein bound to the alpha (2) macroglobulin receptor, or fragment, analog, derivative or mimetic thereof, such that if the amount of bound heat shock protein measured in (b) differs from the amount of bound heat shock protein measured in the absence of the test compound, then a compound that modulates the binding of an HSP to the  $\alpha$ 2M receptor is identified.

18. The method of Claim 65 wherein the solid surface is a microtiter dish.

19. The method of Claim 17 wherein the amount of bound heat shock protein is measured by contacting the cell with a heat shock protein-specific antibody.

20. The method of Claim 17 wherein the heat shock protein is labeled and the amount of bound heat shock protein is measured by detecting the label.

21. The method of Claim 20 wherein the heat shock protein is labeled with a fluorescent label.

22. A method for identifying a compound that modulates heat shock protein-mediated antigen presentation by alpha (2) macroglobulin receptor-expressing cells comprising:

- (a) adding a test compound to a mixture of alpha (2) macroglobulin receptor-expressing cells and a complex consisting essentially of a heat shock protein noncovalently associated with an antigenic molecule, under conditions conducive to alpha (2) macroglobulin receptor-mediated endocytosis;
- (b) measuring the level of stimulation of antigen-specific cytotoxic T cells by the alpha (2) macroglobulin receptor-expressing cells, such that if the level measured in (b) differs from the level of said stimulation in the absence of the test compound, then a compound that modulates heat shock protein-mediated antigen presentation by alpha (2) macroglobulin receptor-expressing cells is identified.

23. A method for detecting a heat shock protein-alpha (2) macroglobulin receptor-related disorder in a mammal comprising measuring the level of activity from an HSP-alpha (2) macroglobulin receptor-mediated process in a patient sample, such that if the measured level differs from the level found in clinically normal individuals, then a heat shock protein-alpha (2) macroglobulin receptor-related disorder is detected.

24. The method of Claim 23 comprising contacting a sample derived from a patient with an antibody specific for the alpha (2) macroglobulin receptor under conditions such that immunospecific binding by the antibody.
- 5 25. The method of Claim 23 comprising contacting a sample derived from a patient with an antibody specific for a heat shock protein under conditions such that immunospecific binding by the antibody.
- 10 26. The method of Claim 23 comprising contacting a sample derived from a patient with an antibody specific for an HSP- $\alpha$ 2M complex under conditions such that immunospecific binding by the antibody.
- 15 27. A method for modulating an immune response comprising administering to a mammal a purified compound that modulates the interaction of a heat shock protein with the alpha (2) macroglobulin receptor.
- 20 28. The method of Claim 27, in which the compound is an agonist which enhances the interaction of the heat shock protein and the alpha (2) macroglobulin receptor.
- 25 29. A method for treating an autoimmune disorder comprising administering to a mammal in need of such treatment a purified compound that interferes with the interaction of a heat shock protein with the alpha (2) macroglobulin receptor.
- 30 30. The method of Claim 29 in which the compound is an antagonist that interferes with the interaction between the heat shock protein and the  $\alpha$ 2M receptor.
- 35 31. The method of Claim 30, in which the antagonist is an antibody specific for alpha (2) macroglobulin receptor.
32. The method of Claim 30, in which the antagonist is an antibody specific for a heat shock protein.
33. The method of Claim 30, in which the antagonist is a small molecule.
34. The method of Claim 30, in which the antagonist is a peptide.
- 35 35. The method of Claim 30, in which the peptide comprises at least 5

consecutive amino acids of alpha (2) macroglobulin receptor (SEQ ID NO.:1).

36. The method of Claim 30, in which the peptide comprises at least 5 consecutive amino acids of alpha (2) macroglobulin (SEQ ID NO.: 3).

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37. The method of Claim 30, in which the peptide comprises at least 5 consecutive amino acids of a heat shock protein sequence.

38. A method for treating an autoimmune disorder comprising administering to a mammal in need of such treatment a recombinant cell that expresses an alpha (2) macroglobulin receptor which decreases the uptake of a heat shock protein by a functional alpha (2) macroglobulin receptor.

39. A method for increasing the immunopotency of a cancer cell or an infected cell comprising transforming said cell with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin receptor polypeptide.

40. A method for increasing the immunopotency of a cancer cell or an infected cell comprising:

- (a) transforming said cell with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin receptor polypeptide, and
- (b) administering said cell to an individual in need of treatment, so as to obtain an elevated immune response.

41. A recombinant cancer cell transformed with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin receptor polypeptide.

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42. A recombinant infected cell transformed with a nucleic acid comprising a nucleotide sequence that (i) is operably linked to a promoter, and (ii) encodes an alpha (2) macroglobulin receptor polypeptide.

43. The recombinant cell of Claim 41 or 42 which is a human cell.

35

44. A kit, comprising in one or more containers: (a) an anti- $\alpha$ 2M receptor

antibody or a nucleic acid probe capable of hybridizing to an  $\alpha$ 2M receptor nucleic acid, (b) a purified heat shock protein, nucleic acid encoding a heat shock protein, or cell expressing a heat shock protein; and (c) instructions for use in detecting a heat shock protein-alpha (2) macroglobulin receptor-related disorder.

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45. The kit of Claim 44 wherein the antibody or nucleic acid probe is labeled with a detectable marker.

46. The kit of Claim 44 further comprising a labeled macroglobulin receptor  
10 polypeptide.

47. A kit, in one or more containers, comprising: (a) a purified heat shock protein, nucleic acid encoding a heat shock protein, or cell expressing a heat shock protein; and (b) an alpha (2) macroglobulin receptor polypeptide, nucleic acid encoding an alpha (2)  
15 macroglobulin receptor polypeptide, or cell expressing an alpha (2) macroglobulin receptor polypeptide.

48. The kit of Claim 47 in which the alpha (2) macroglobulin receptor polypeptide, nucleic acid encoding an alpha (2) macroglobulin receptor polypeptide, or cell  
20 expressing an alpha (2) macroglobulin receptor polypeptide is purified.

49. The kit of Claim 47 further comprising instructions for use in treating an autoimmune disorder, an infectious disease, or a proliferative disorder.

25 50. A method for identifying an  $\alpha$ 2M receptor fragment capable of binding a heat shock protein, said method comprising:

- (a) contacting a heat shock protein, or peptide-binding fragment thereof, with one or more alpha (2) macroglobulin receptor fragments; and
- (b) identifying an  $\alpha$ 2M receptor fragment which specifically binds to the heat  
30 shock protein, or peptide-binding fragment thereof.

51. A method for identifying an  $\alpha$ 2M receptor fragment capable of inducing an HSP- $\alpha$ 2M receptor-mediated process, said method comprising:

- (a) contacting a heat shock protein with a cell expressing  $\alpha$ 2M receptor fragment;  
35 and
- (b) measuring the level of alpha (2) macroglobulin receptor activity in the cell, such that if the level of the HSP- $\alpha$ 2M receptor-mediated process or activity measured in (b)

is greater than the level of alpha (2) macroglobulin receptor activity in the absence of the  $\alpha$ 2M receptor fragment, then an  $\alpha$ 2M receptor fragment capable of inducing an HSP- $\alpha$ 2M receptor-mediated process is identified.

5           52.     The method of Claim 51 wherein the alpha (2) macroglobulin receptor activity measured is the ability to interact with the heat shock protein.

          53.     The method of Claim 51 wherein the heat shock protein is non-covalently associated with an antigenic peptide and the alpha (2) macroglobulin receptor activity  
10 measured is the ability to re-present the antigenic peptide.

          54.     The method of Claim 51 wherein the heat shock protein is non-covalently associated with an antigenic peptide and the alpha (2) macroglobulin receptor activity measured is the ability to stimulate a cytotoxic T cell response against the antigenic peptide.

15

          55.     A method for identifying a heat shock protein fragment capable of binding an  $\alpha$ 2M receptor, said method comprising:

- (a)     contacting an  $\alpha$ 2M receptor with one or more heat shock protein fragments;  
          and
- 20       (b)     identifying a heat shock protein fragment which specifically binds to the  $\alpha$ 2M receptor.

          56.     A method for identifying a heat shock protein fragment capable of inducing an HSP- $\alpha$ 2M receptor-mediated process, said method comprising:

- 25       (a)     contacting an  $\alpha$ 2M receptor fragment with a cell expressing a heat shock protein; and
- (b)     measuring the level of alpha (2) macroglobulin receptor activity in the cell, such that if the level of the HSP- $\alpha$ 2M receptor-mediated process or activity measured in (b) is greater than the level of alpha (2) macroglobulin receptor activity in the absence of said  
30 heat shock protein fragment, then a heat shock protein fragment capable of inducing an HSP- $\alpha$ 2M receptor-mediated process is identified.

          57.     The method of Claim 56 wherein the alpha (2) macroglobulin receptor activity measured is the ability to interact with the heat shock protein fragment.

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          58.     The method of Claim 56 wherein the heat shock protein fragment is non-covalently associated with an antigenic peptide and the alpha (2) macroglobulin receptor

activity measured is the ability to re-present the antigenic peptide.

59. The method of Claim 56 wherein the heat shock protein fragment is non-covalently associated with an antigenic peptide and the alpha (2) macroglobulin receptor activity measured is the ability to stimulate a cytotoxic T cell response against the antigenic peptide.

60. A method for identifying a molecule that binds specifically to an  $\alpha$ 2M receptor, said method comprising:

- 10 (a) contacting an  $\alpha$ 2M receptor with one or more test molecules under conditions conducive to binding; and
- (b) identifying one or more test molecules that specifically bind to the  $\alpha$ 2M receptor.

61. The method of Claim 60 wherein said test molecules are potential immunotherapeutic drugs.

62. A method for screening for molecules that specifically bind to an  $\alpha$ 2M receptor comprising:

- 20 (a) contacting an  $\alpha$ 2M receptor with one or more test molecules under conditions conducive to binding; and
- (b) determining whether any of said test molecules specifically bind to the  $\alpha$ 2M receptor.

63. A method for identifying a compound that modulates the binding of an  $\alpha$ 2M receptor ligand to the  $\alpha$ 2M receptor comprising:

- 25 (a) contacting an  $\alpha$ 2M receptor with an  $\alpha$ 2M receptor ligand, or an  $\alpha$ 2M receptor-binding fragment, analog, derivative or mimetic thereof, in the presence of one or more test compounds; and
- 30 (b) measuring the amount of  $\alpha$ 2M receptor ligand, or fragment, analog, derivative or mimetic thereof, bound to the  $\alpha$ 2M receptor,

such that if the amount of bound  $\alpha$ 2M receptor ligand measured in (b) differs from the amount of bound  $\alpha$ 2M receptor measured in the absence of the test compound, then a compound that modulates the binding of an  $\alpha$ 2M receptor ligand to the  $\alpha$ 2M receptor is identified.

64. The method of Claim 17 or 63, in which the alpha (2) macroglobulin receptor

contacted in step (a) is on a cell surface.

65. The method of Claim 17 or 63, wherein the alpha (2) macroglobulin receptor is immobilized to a solid surface.

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66. The method of Claim 1, 64, or 22 in which the heat shock protein is gp96.

67. The method of Claim 1, 64, or 22 in which the heat shock protein is hsp90.

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68. The method of Claim 1, 64, or 22 in which the heat shock protein is hsp70.

69. The method of Claim 1, 64, or 22 in which the heat shock protein is calreticulin.

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70. A method for identifying a compound that modulates the interaction between the  $\alpha$ 2M receptor and an  $\alpha$ 2M receptor ligand, comprising:

(a) contacting an  $\alpha$ 2M receptor with one or more test compounds; and

(b) measuring the level of  $\alpha$ 2M receptor activity or expression,

such that if the level of activity or expression measured in (b) differs from the level of  $\alpha$ 2M receptor activity in the absence of one or more test compounds, then a compound that modulates the interaction between the  $\alpha$ 2M receptor and an  $\alpha$ 2M receptor ligand is identified.

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71. The method of Claim 63 or 70 wherein the  $\alpha$ 2M receptor ligand is  $\alpha$ 2 macroglobulin.

25

72. A method for identifying a compound that modulates antigen presentation by  $\alpha$ 2M receptor-expressing cells comprising:

(a) adding one or more test compounds to a mixture of  $\alpha$ 2M receptor-expressing cells and a complex comprising an  $\alpha$ 2M receptor ligand and an antigenic

30

molecule, under conditions conducive to  $\alpha$ 2M receptor-mediated endocytosis;  
(b) measuring the level of stimulation of antigen-specific cytotoxic T cells by the  $\alpha$ 2M receptor-expressing cells,

such that if the level measured in (b) differs from the level of said stimulation in the absence of the one or more test compounds, then a compound that modulates antigen presentation by  $\alpha$ 2M receptor-expressing cells is identified.

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73. The method of Claim 22 or 72, in which the measuring stimulation of antigen-

specific cytotoxic T cells by the  $\alpha$ 2M receptor-expressing cells of step (b) comprises:

- (i) adding the alpha (2) macroglobulin receptor-expressing cells formed in step (a) to T cells under conditions conducive to the activation of the T cells; and
- 5 (ii) comparing the level of activation of said cytotoxic T cells with the level of activation of T cells by an alpha (2) macroglobulin receptor-expressing cell formed in the absence of the test compound,

wherein an increase or decrease in level of T cell activation indicates that a compound that modulates heat shock protein-mediated antigen presentation by alpha (2) macroglobulin  
10 receptor-expressing cells is identified.

74. A method for modulating an immune response comprising administering to a mammal a purified compound that binds to the  $\alpha$ 2M receptor, in an amount effective to modulate an immune response in the mammal.

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75. A method for treating or preventing a disease or disorder comprising administering to a mammal a purified compound that binds to the  $\alpha$ 2M receptor, in an amount effective to treat or prevent the disease or disorder in the mammal.

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76. The method of Claim 75 wherein the disease or disorder is cancer or an infectious disease.

77. A method for treating an autoimmune disorder comprising administering to a mammal in need of such treatment a purified compound that binds to the  $\alpha$ 2M receptor, in an  
25 amount effective to treat an autoimmune disorder in the mammal.

78. A method for stimulating an immune response in a patient comprising administering to said patient blood which has been withdrawn from said patient and treated to remove an  $\alpha$ 2M receptor ligand.

30

79. The method of Claim 78 further comprising administering to said patient a heat shock protein or a heat shock protein-antigenic peptide complex.

80. A method for stimulating an immune response in a patient comprising:  
35 (a) removing a  $\alpha$ 2M receptor ligand from blood withdrawn from said patient; and  
(b) returning at least a portion of the  $\alpha$ 2M receptor ligand-depleted blood to said patient.

81. A method for stimulating an immune response in a patient comprising:
- (a) withdrawing blood from said patient;
  - (b) removing a  $\alpha$ 2M receptor ligand from said blood; and
  - (c) returning at least a portion of the  $\alpha$ 2M receptor ligand-depleted blood to said patient.
- 5
82. The method of Claim 81 further comprising after step (a) and before step (c) the step of adding a heat shock protein or a heat shock protein-antigenic peptide complex to said blood.
- 10
83. The method of Claims 80 or 81 wherein removing a  $\alpha$ 2M receptor ligand from the blood comprises the step of contacting the blood with a solid phase attached to a  $\alpha$ 2M receptor ligand-binding molecule for a time period and under conditions sufficient to allow binding of  $\alpha$ 2M receptor ligand to the  $\alpha$ 2M receptor ligand-binding molecule solid phase.
- 15
84. The method of Claim 83 wherein the  $\alpha$ 2M receptor ligand-binding molecule is  $\alpha$ 2M receptor, or a fragment thereof.
85. The method of Claim 83 wherein said  $\alpha$ 2M receptor ligand-binding molecule
- 20 does not bind a heat shock protein.
86. The method of Claim 85 wherein the  $\alpha$ 2M receptor ligand-binding molecule is an  $\alpha$ 2M receptor ligand-specific antibody, or a fragment thereof.
- 25
87. The method of Claims 80 or 81 wherein an apheresis system is used in said removing step.
88. The method of Claim 81 wherein blood is withdrawn manually in said withdrawing step.
- 30
89. The method of Claim 80 or 81 wherein said removing step comprises separating the plasma from said blood and treating said plasma to remove said  $\alpha$ 2M receptor ligand.
- 35
90. The method of Claim 78 wherein said blood is administered to said patient by syringe.

91. The method of Claim 78 wherein said blood is administered to said patient by an intravenous drip.

5 92. The method of Claim 80 or 81 wherein said blood is returned to said patient by syringe.

93. The method of Claim 80 or 81 wherein said blood is returned to said patient by an intravenous drip.

10 94. A kit comprising in one or more containers a solid phase chromatography column with a purified  $\alpha$ 2M receptor ligand binding molecule attached thereto, such that withdrawn blood can be run over the column to deplete the blood of a  $\alpha$ 2M receptor ligand.

15 95. The kit of Claim 94 wherein the  $\alpha$ 2M receptor ligand binding molecule does not bind heat shock proteins.

20 96. The method of Claim 78, 80, or 81 wherein the  $\alpha$ 2M receptor ligand is  $\alpha$ 2M, a lipoprotein complex, lactoferrin, tissue-type plasminogen activator, urokinase-type plasminogen activator, or an exotoxin.

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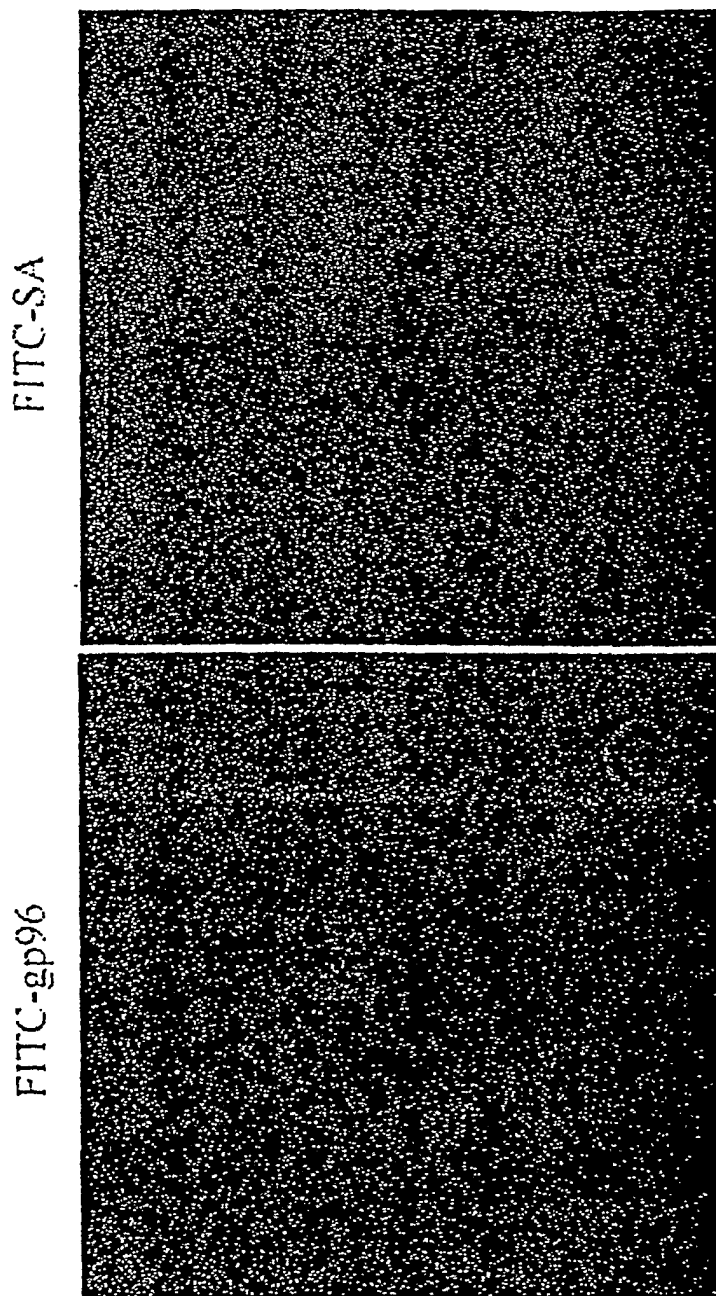


FIG. 1a

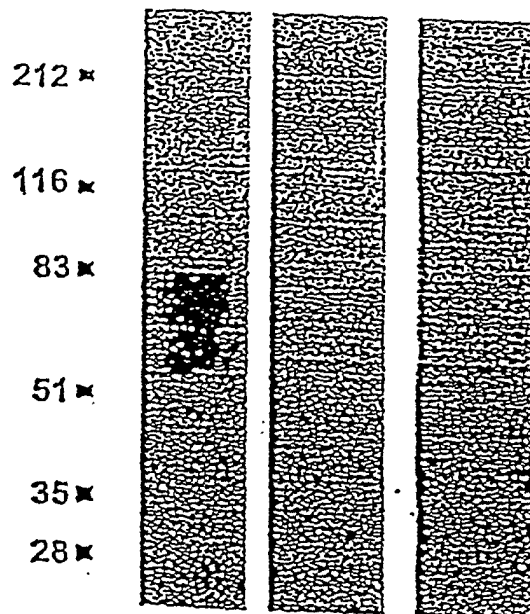
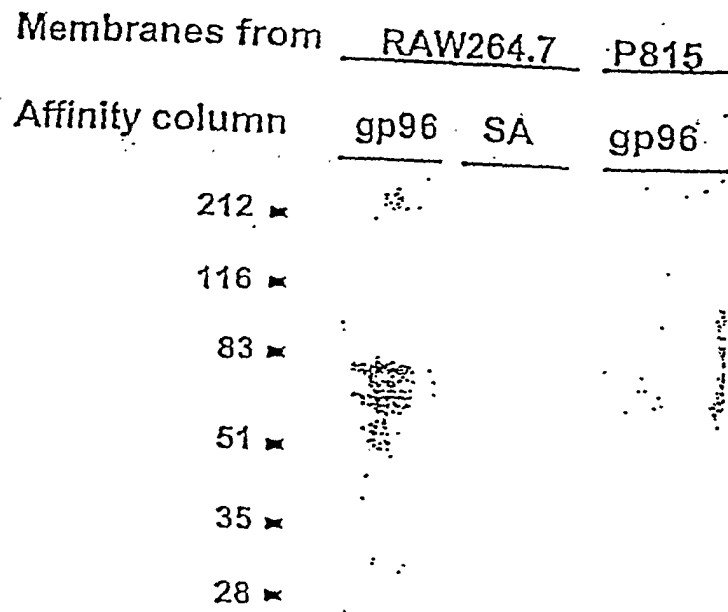


FIG. 1b

Cells	MO	MO	MO	P815
<sup>125</sup> I-SASD-gp96	+	+	+	+
UV	+	-	+	+
2-ME	+	+	-	+

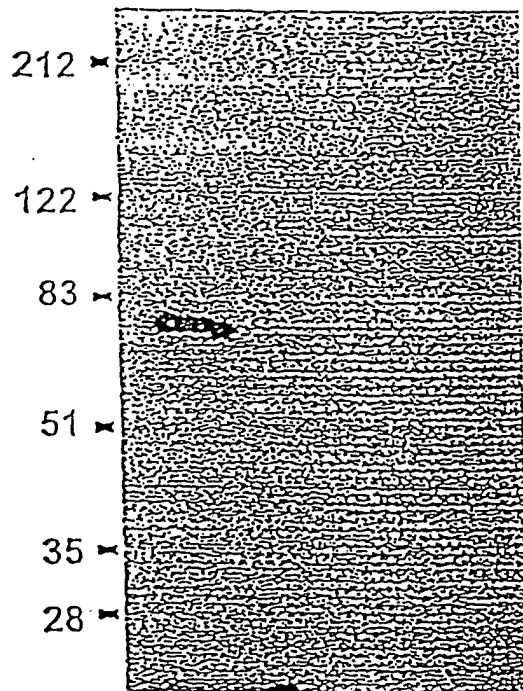


FIG. 1c

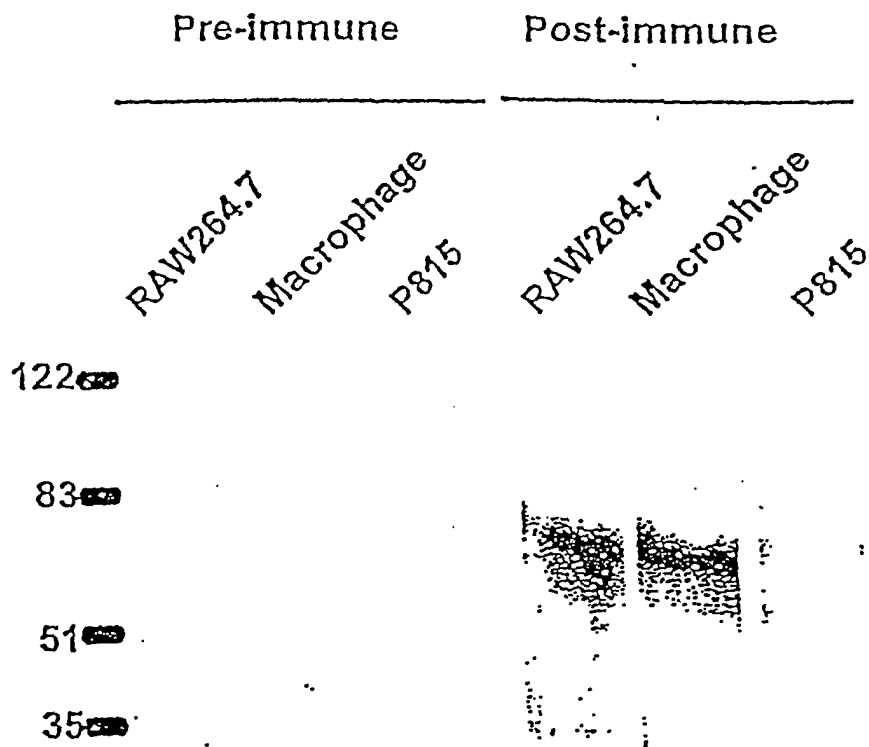


FIG. 2a

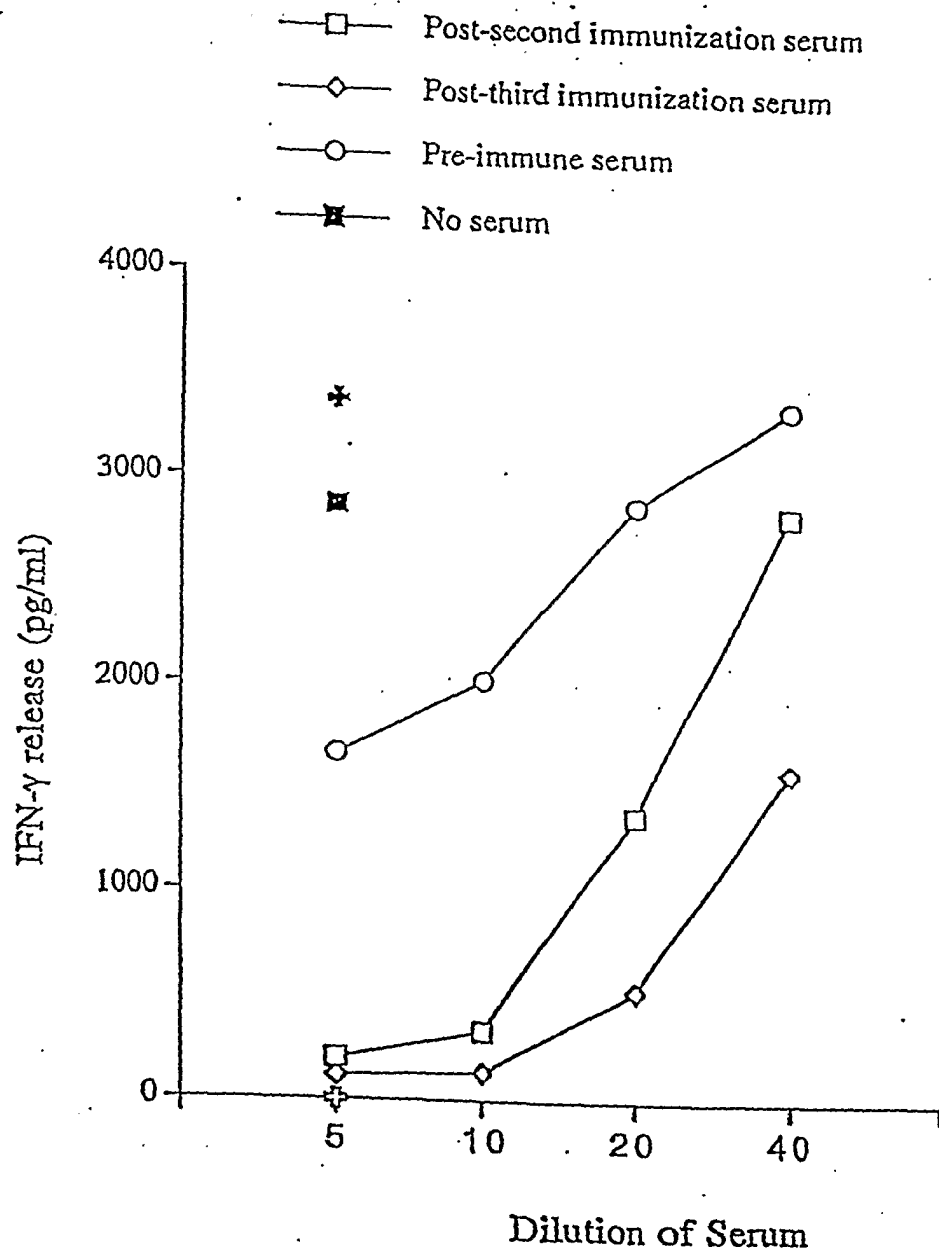


FIG. 2b

Seq #	b	y	+1
G 1	58.1	-	10
G 2	115.1	1095.2	9
A 3	186.2	1038.2	8
L 4	299.3	967.1	7
H 5	436.5	853.9	6
I 6	549.6	716.8	5
Y 7	712.8	603.6	4
H 8	850.0	440.5	3
Q 9	978.1	303.3	2
R 10	-	175.2	1

FIG. 3a

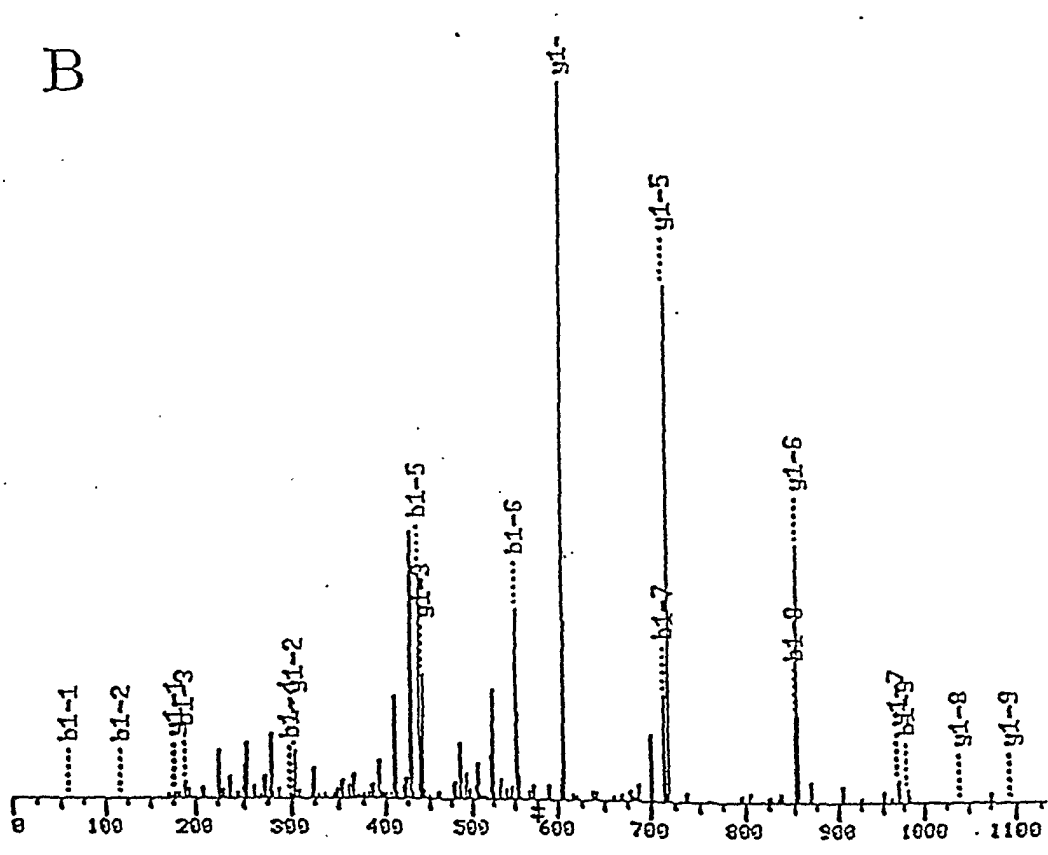


FIG. 3b

Position	MH+	Sequence
509-518	955.0122	SGFSLGSDGK (Seq 10 no: 54)
328-337	973.1753	GIALDPAMGK (Seq 10 no: 55)
460-469	1152.3010	GGALHIYHQR (Seq 10 no: 56)
338-348	1315.5116	VFFTDYGQIPK (Seq 10 no: 57)

FIG. 3c

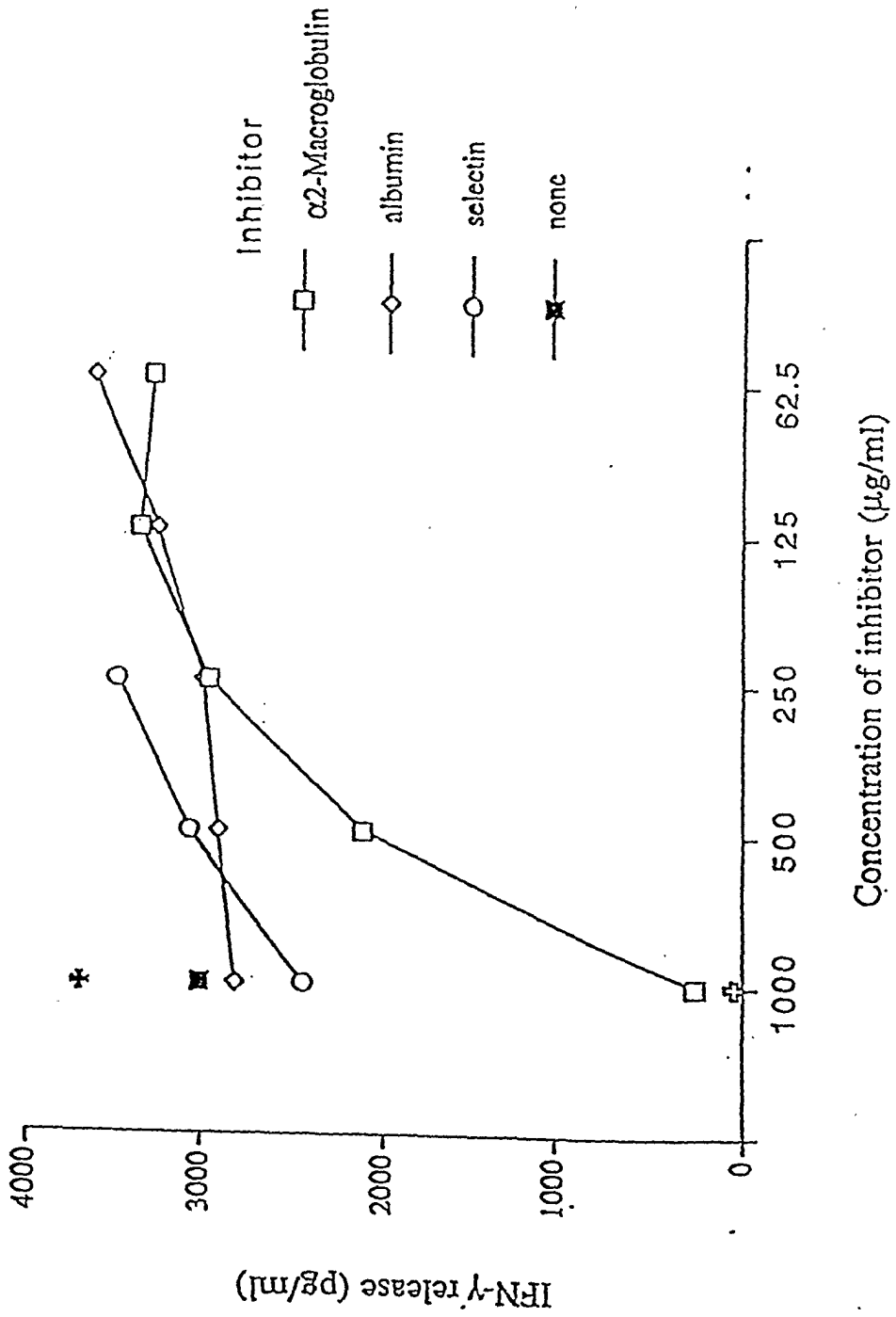


FIG. 4

**Table 1. Specific binding of HSPs and  $\alpha_2$ -macroglobulin to primary cultures and cell lines of several histological origins\***

Cells	Cell type	Haplotype	**% cells binding with FITC-labeled:				
			$\alpha_2M$	gp96	hsp70	hsp90	SA
B16	Melanoma	<i>b</i>	0.1	3.5	6.4	8.0	0.3
CT26	Carcinoma	<i>d</i>	N/D	0.3	3.1	5.5	0.4
YAC-1	Lymphoma	<i>b</i>	0.1	3.1	23.0	5.0	0.2
EL4	T cell thymoma	<i>b</i>	0.1	2.9	3.0	6.6	1.0
Meth A	Sarcoma	<i>d</i>	0.1	0.1	1.5	0.9	0.5
PS-C3H	Fibrosarcoma	<i>k</i>	0.1	0.1	2.0	0.3	0.3
UV6139	Sarcoma	<i>k</i>	11	0.0	0.7	0.2	1.5
P815	Mastocytoma	<i>d</i>	0.1	1.1	1.7	0.7	0.2
Peritoneal cells	Macrophage	<i>d</i>	90	97	82	82	11
BM-DCs	Dendritic cells	<i>b</i> and <i>d</i>	+++ <sup>#</sup>	+++	+++	+++	-
RAW264.7 <sup>*</sup>	Macrophage	<i>d</i>	76	82	85	90	8.0
RAW309Cr.1 <sup>*</sup>	Macrophage	<i>b x d</i>	0.1	0.1	0.1	0.1	0.1

**FIG. 5**

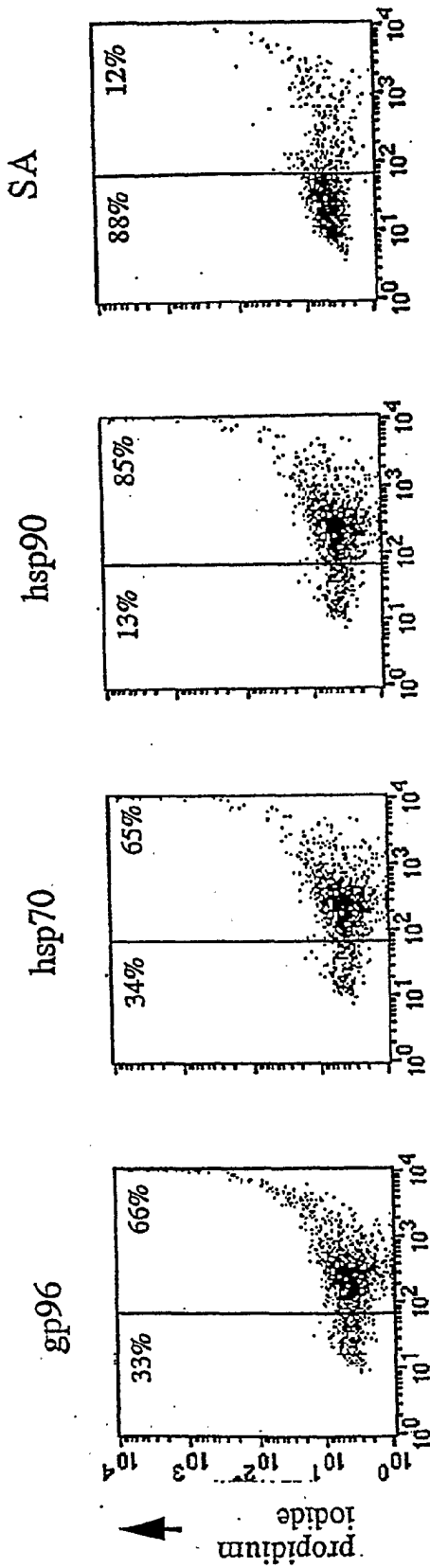


FIG. 6A

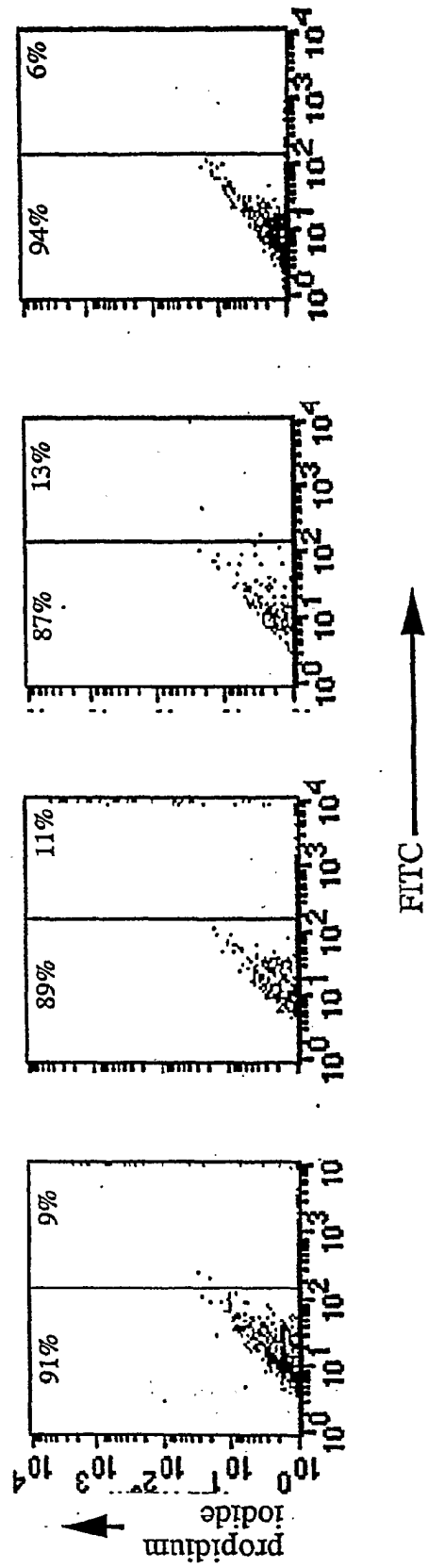
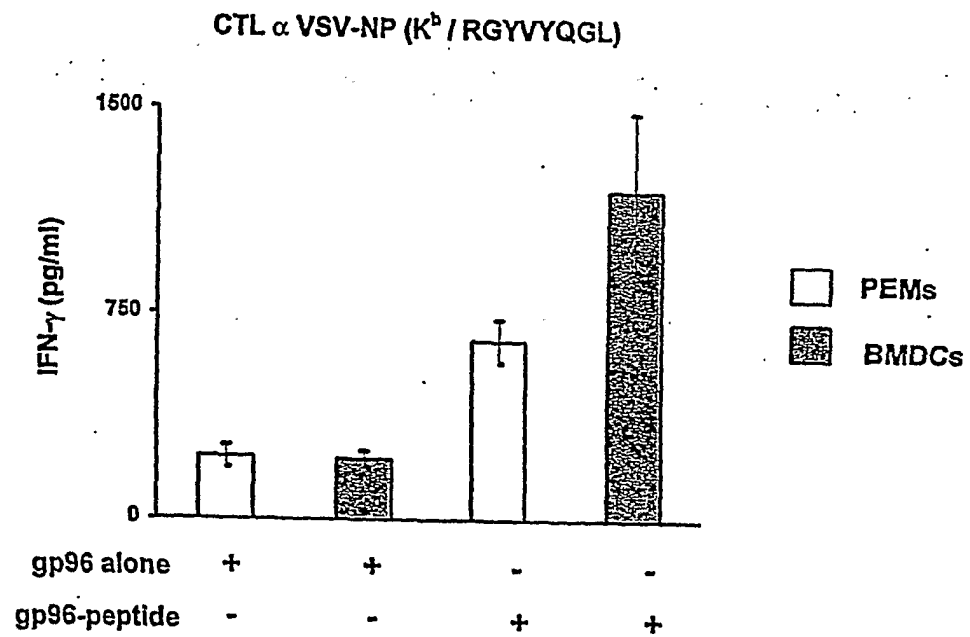
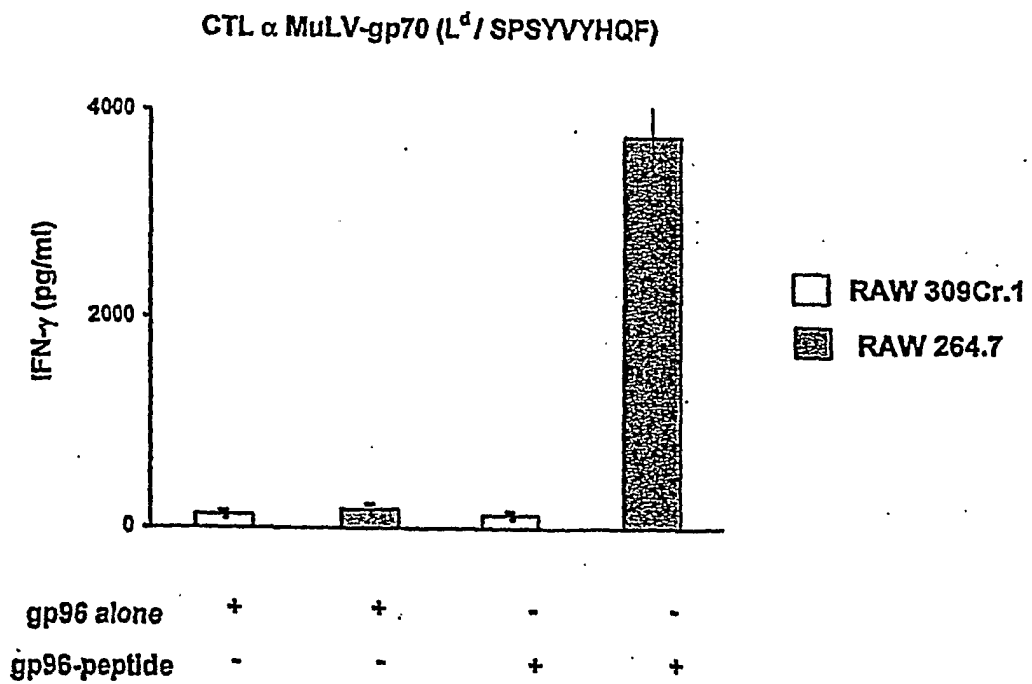


FIG. 6B



**FIG. 7A**



**FIG. 7B**

CTL  $\alpha$  MuLV-gp70 (L<sup>d</sup>SPSYVYHQF)  
 APC : RAW 264.7

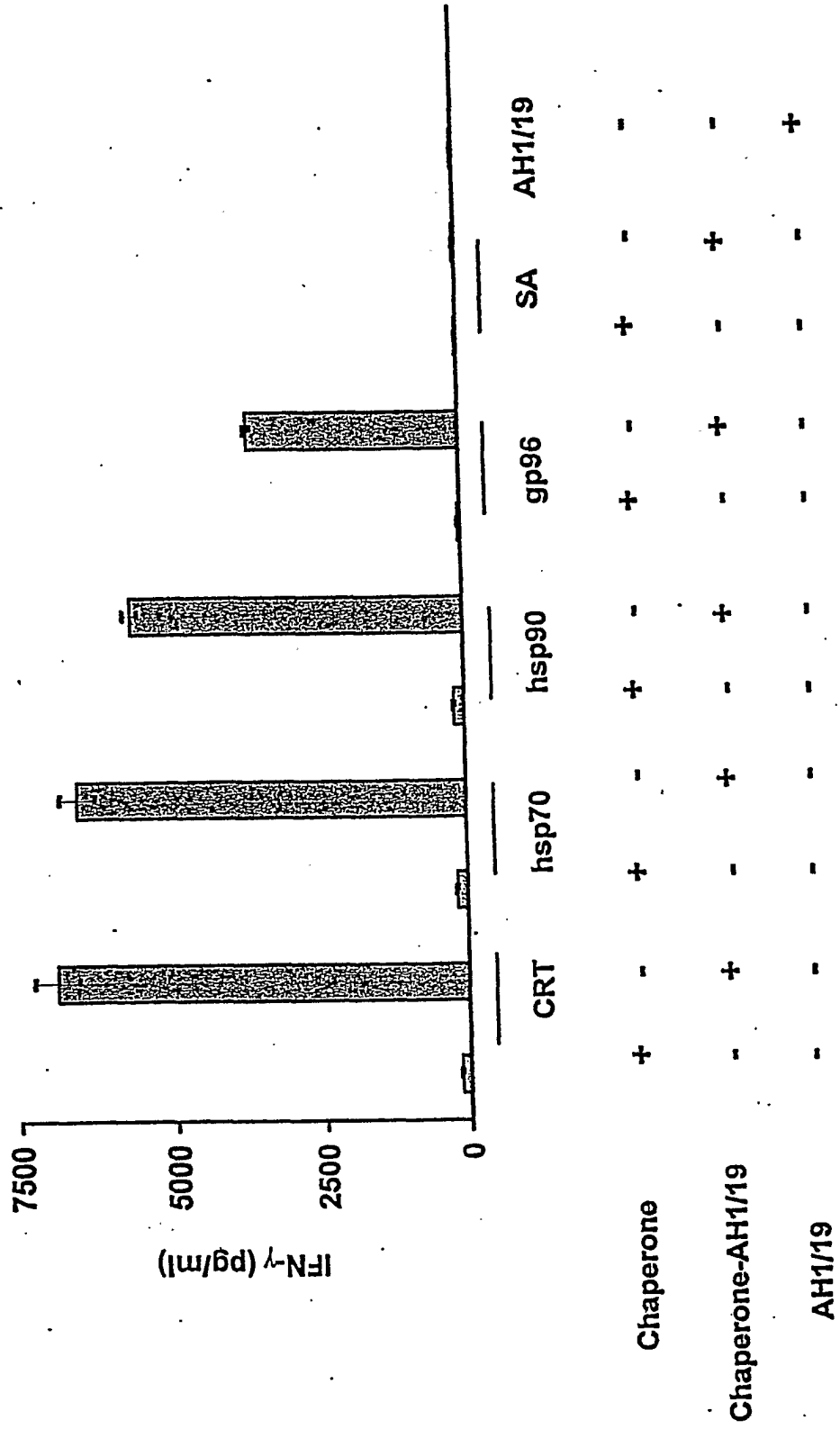


FIG. 8

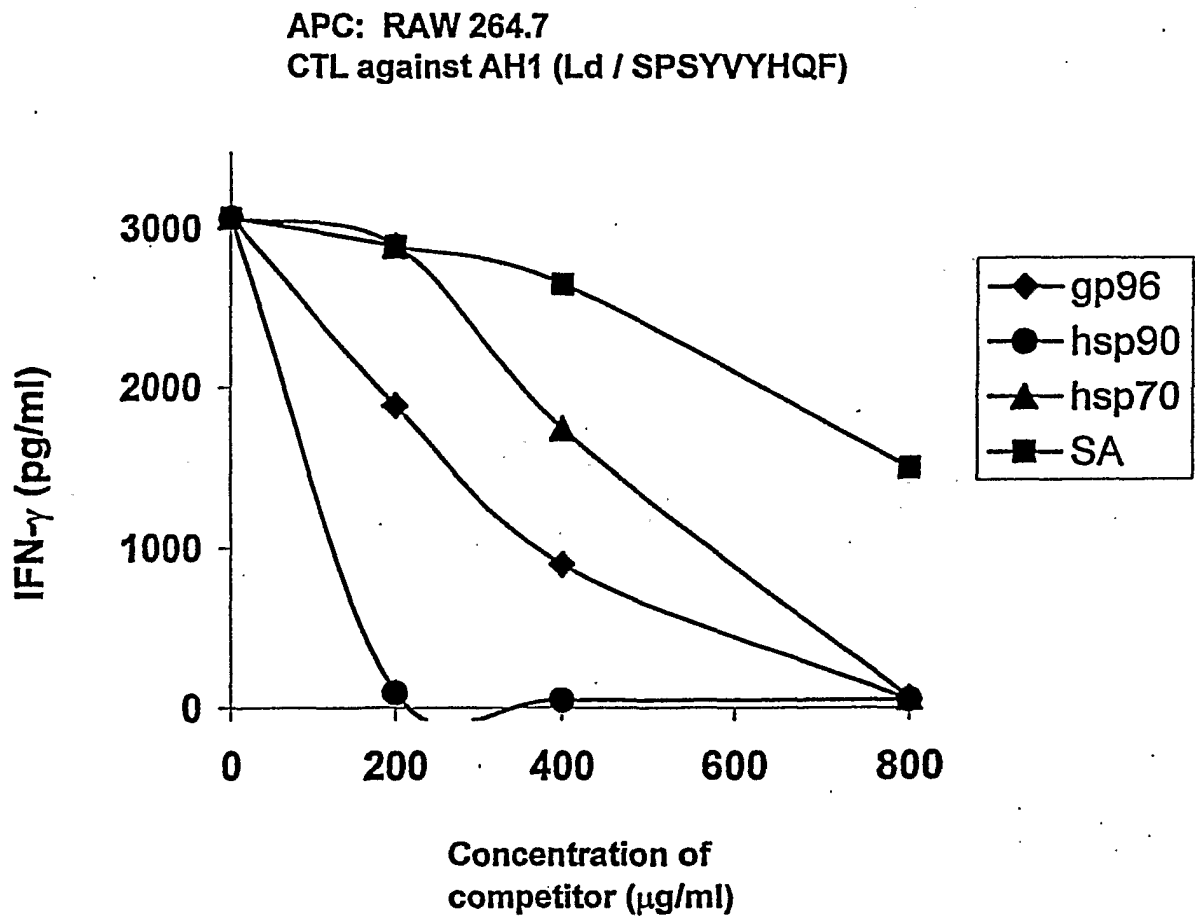


FIG. 9A

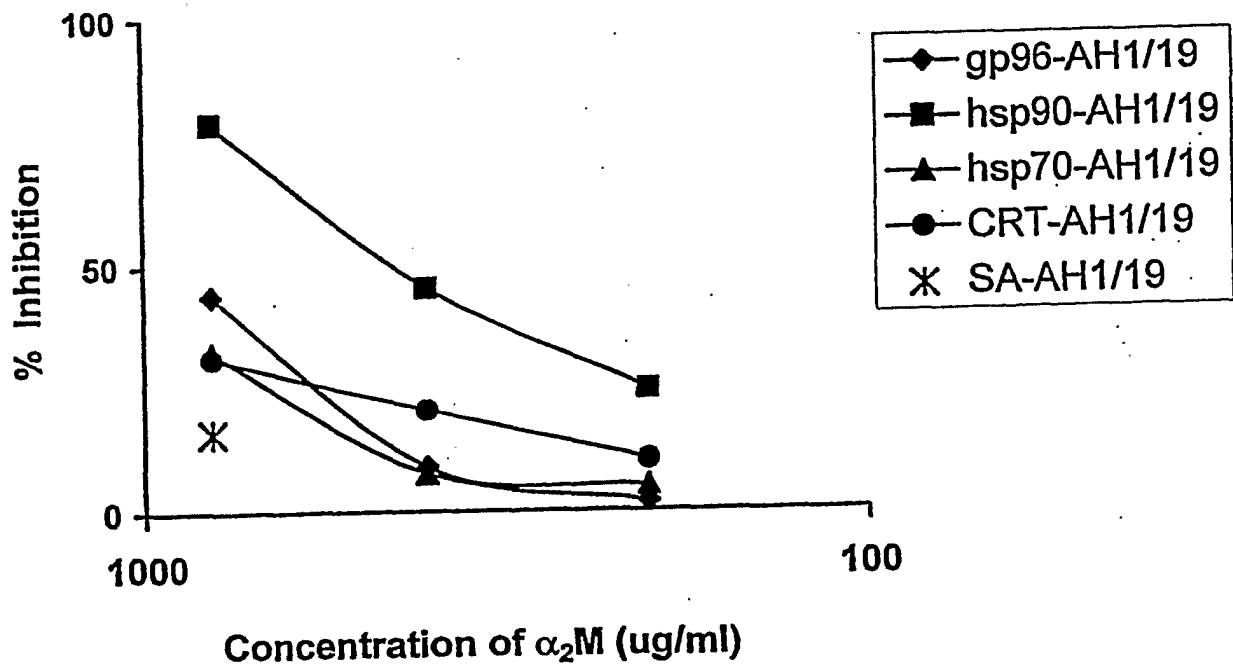


FIG. 9B

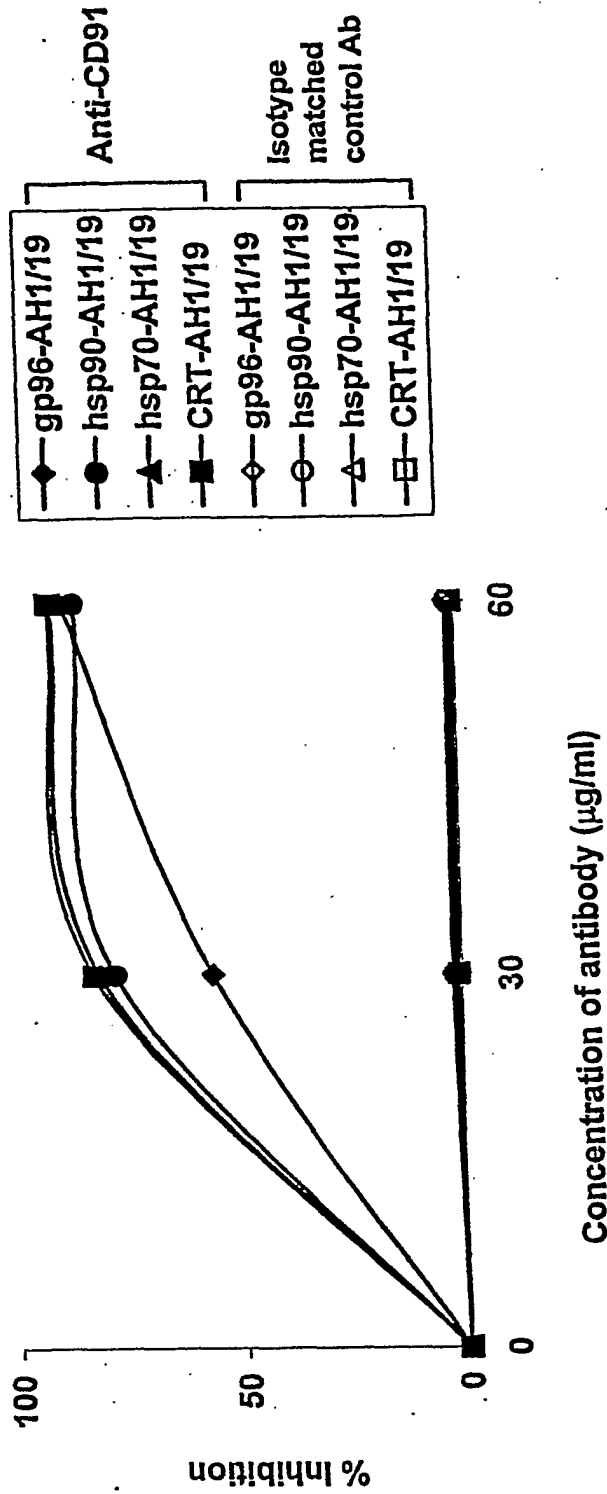


FIG. 9C

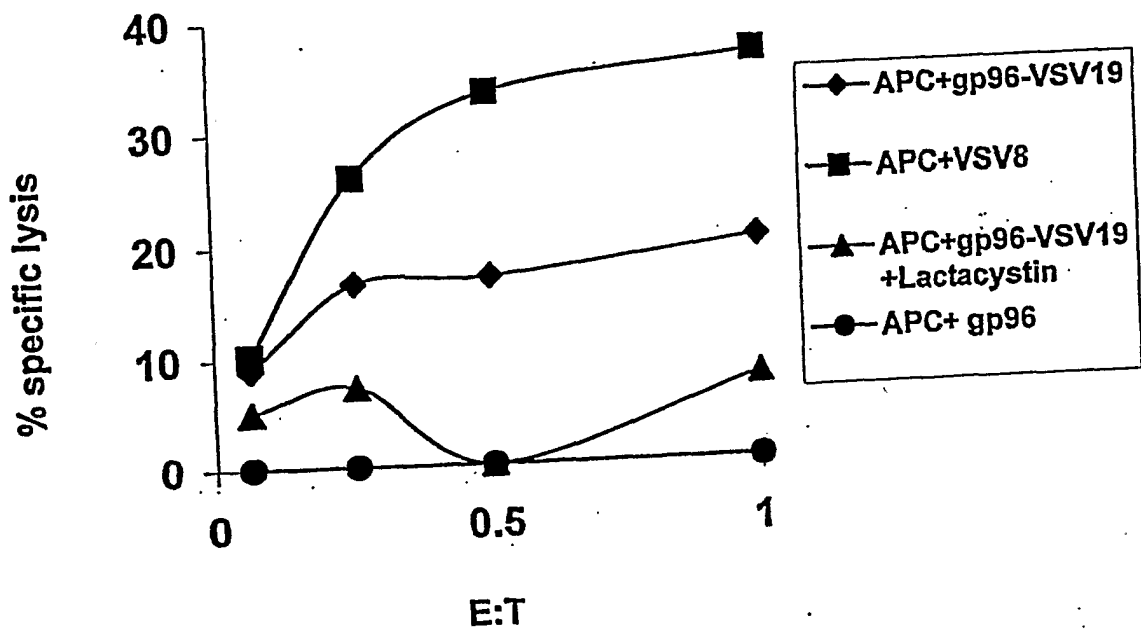


FIG. 10A

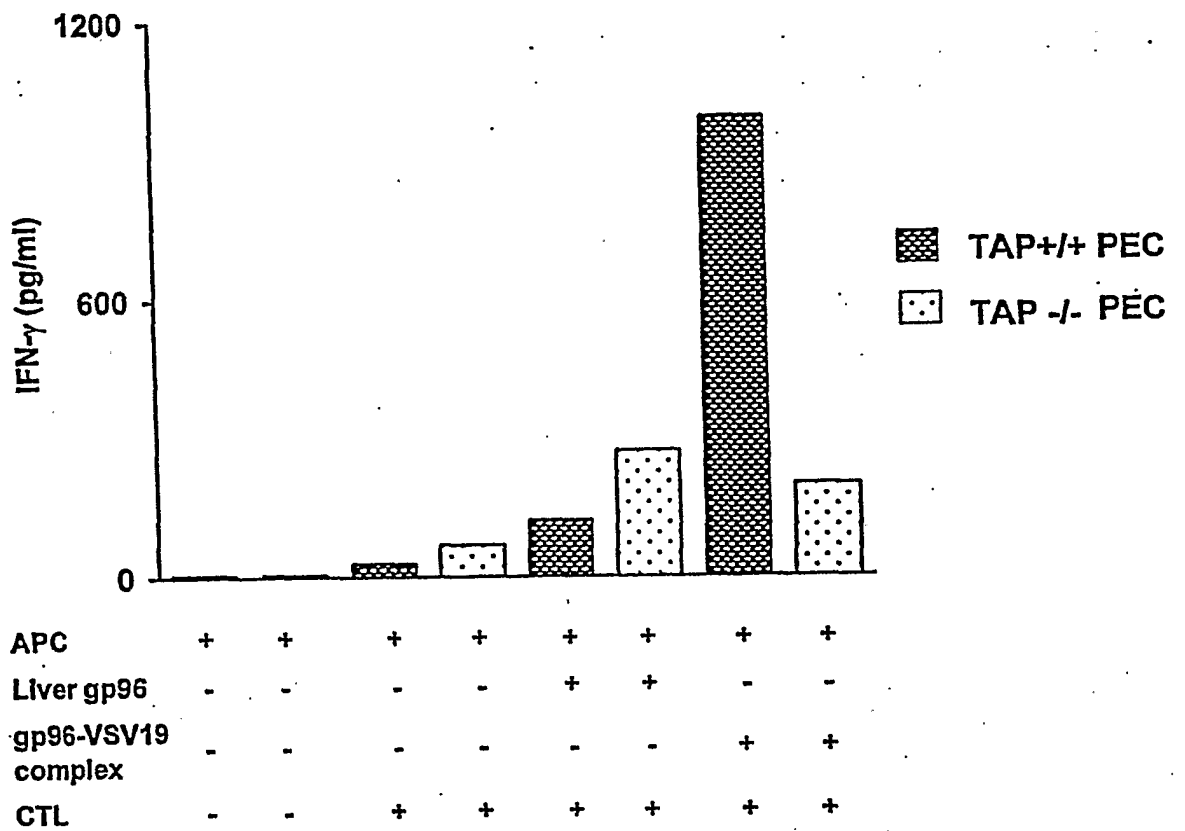


FIG. 10B

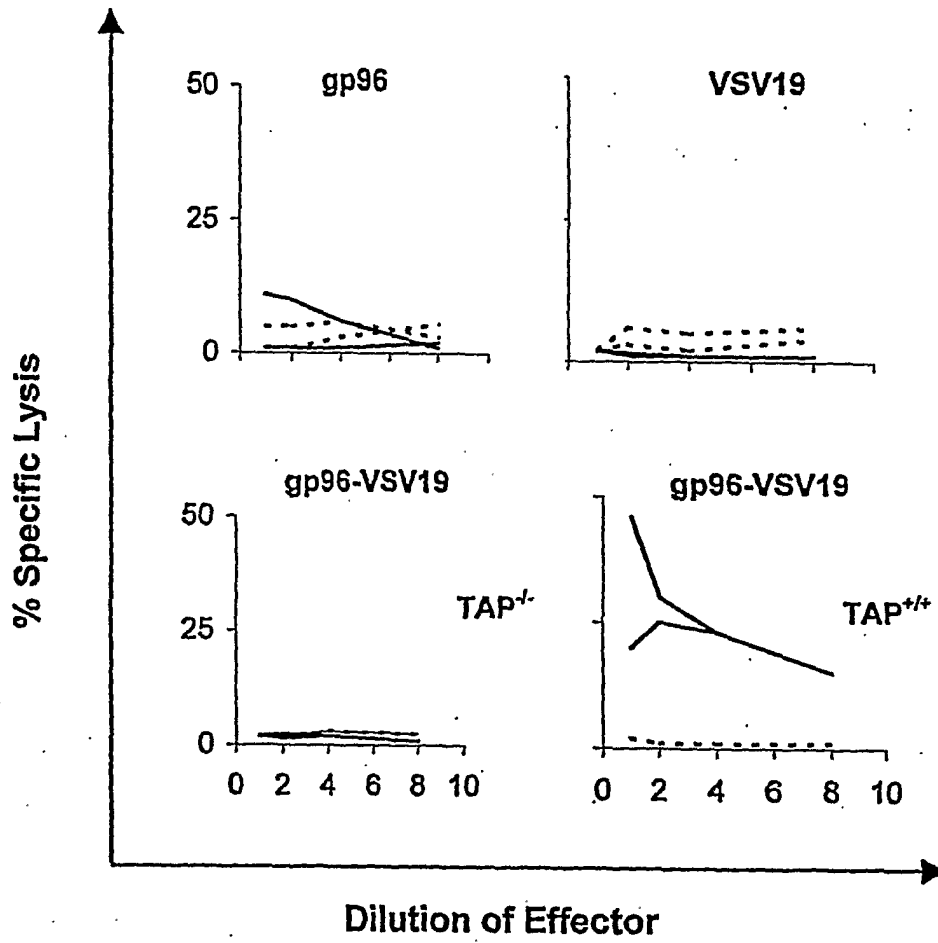


FIG. 10C

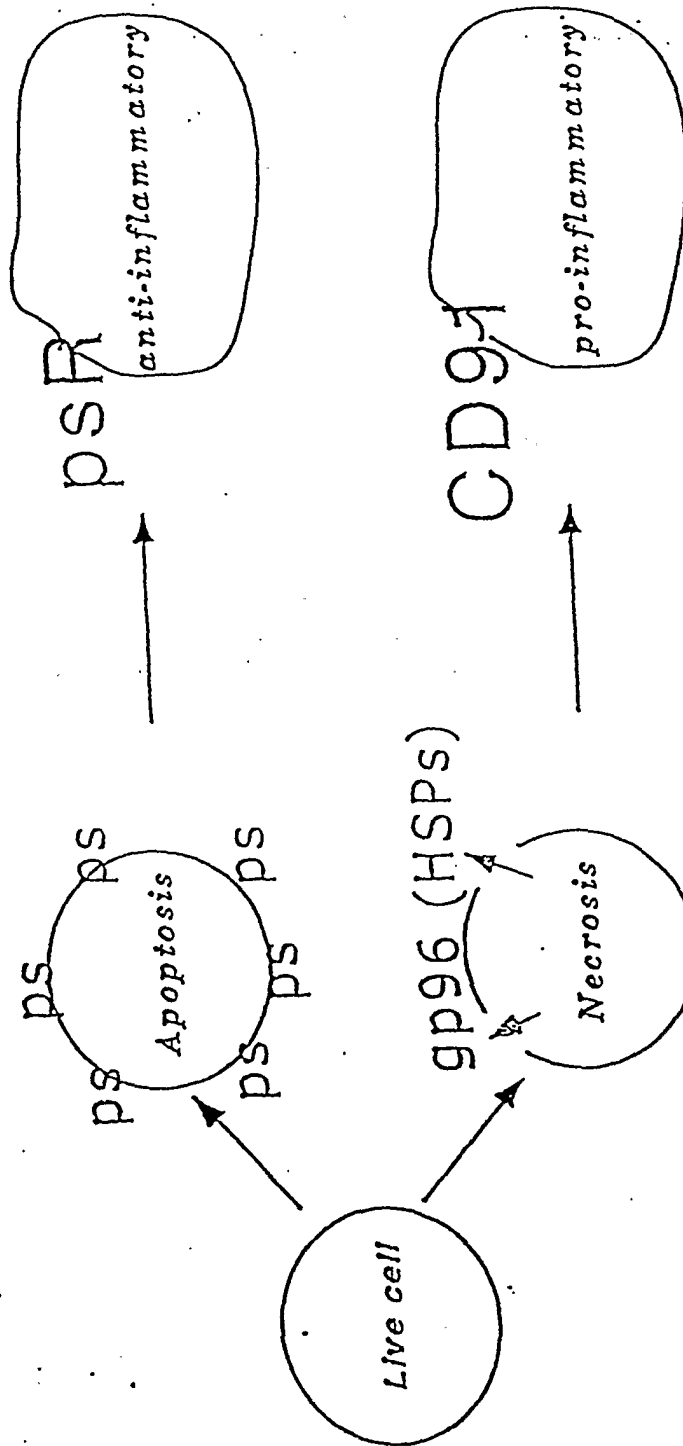


FIG. 11

CGCTGCTCCC	CGCCAGTGCA	CTGAGGAGGC	GGAAACGGGG	GAGCCCCTAG	TGCTCCATCA		60
GGCCCCTACC	AAGGCACCCC	CATCGGGTCC	ACGCCCCCCA	CCCCCACC	CGCTCCTCC		120
CAATTGTGCA	TTTTTGACG	CGGAGTCGGC	TCCGAGATGG	GGCTGTGAGC	TTCGCCCTGG		180
GAGGGGGAGA	GGAGCGAGGA	GTAAGCAGG	GGTGAAGGGT	TCGAATTTGG	GGGCAGGGGG		240
CGCACCCGCG	TCAGCAGGCC	CTTCCCAGGG	GGCTCGGAAC	TGTACCATT	CACCTATGCC		300
CCTGGTTCGC	TTTGCTTAAG	GAAGGATAAG	ATAGAAGAGT	CGGGGAGAGG	AAGATAAAGG		360
GGGACCCCC	AATTGGGGGG	GGCGAGGACA	AGAAGTAACA	GGACCAGAGG	GTGGGGGCTG		420
CTGTTTGCAT	CGGCCACAC	C ATG	CTG ACC	CCG CCG	TTG CTG	CTG CTC	GTG
		Met	Leu	Thr	Pro	Pro	Leu
		1			5		10
CGG CTG CTT	TCA GCT CTG	GTC TCC	GGG GCC	ACT ATG	GAT GCC	CCT AAA	519
Pro Leu Leu	Ser Ala Leu	Val Ser	Gly Ala	Thr Met	Asp Ala	Pro Lys	
	15		20		25		
ACT TGC AGC	CCT AAG CAG	TTT GCC	TGC AGA	GAC CAA	ATC ACC	TGT ATC	567
Thr Cys Ser	Pro Lys Gln	Phe Ala	Cys Arg	Asp Gln	Ile Thr	Cys Ile	
	30		35		40		
TCA AAG GGC	TGG CGG TGT	GAC GGT	GAA AGA	GAT TGC	CCC GAC	GGC TCT	615
Ser Lys Gly	Trp Arg Cys	Asp Gly	Glu Arg	Asp Cys	Pro Asp	Gly Ser	
	45		50		55		
GAT GAA GCC	CCT GAG ATC	TGT CCA	CAG AGT	AAA GCC	CAG AGA	TGC CCG	663
Asp Glu Ala	Pro Glu Ile	Cys Pro	Gln Ser	Lys Ala	Gln Arg	Cys Pro	
	60		65		70		
CCA AAT GAG	CAC AGT TGT	CTG GGG	ACT GAG	CTA TGT	GTC CCC	ATG TCT	711
Pro Asn Glu	His Ser Cys	Leu Gly	Thr Glu	Leu Cys	Val Pro	Met Ser	
	75		80		85		
CGT CTC TGC	AAC GGG ATC	CAG GAC	TGC ATG	GAT GGC	TCA GAC	GAG GGT	759
Arg Leu Cys	Asn Gly Ile	Gln Asp	Cys Met	Asp Gly	Ser Asp	Glu Gly	
	95		100		105		
GCT CAC TGC	CGA GAG CTC	CGA GCC	AAC TGT	TCT CGA	ATG GGT	TGT CAA	807
Ala His Cys	Arg Glu Leu	Arg Ala	Asn Cys	Ser Arg	Met Gly	Cys Gln	
	110		115		120		
CAC CAT TGT	GTA CCT ACA	CCC AGT	GGG CCC	ACG TGC	TAC TGT	AAC AGC	855
His His Cys	Val Pro Thr	Pro Ser	Gly Pro	Thr Cys	Tyr Cys	Asn Ser	
	125		130		135		
AGC TTC CAG	CTC GAG GCA	GAT GGC	AAG ACG	TGC AAA	GAT TTT	GAC GAG	903
Ser Phe Gln	Leu Glu Ala	Asp Gly	Lys Thr	Cys Lys	Asp Phe	Asp Glu	
	140		145		150		
TGT TCC GTG	TAT GGC ACC	TGC AGC	CAG CTT	TGC ACC	AAC ACA	GAT GGC	951
Cys Ser Val	Tyr Gly Thr	Cys Ser	Gln Leu	Cys Thr	Asn Thr	Asp Gly	
	155		160		165		
TCC TTC ACA	TGT GGC TGT	GTT GAA	GGC TAC	CTG CTG	CAA CCG	GAC AAC	999
Ser Phe Thr	Cys Gly Cys	Val Glu	Gly Tyr	Leu Leu	Gln Pro	Asp Asn	
	175		180		185		
CGC TCC TGC	AAG GCC AAG	AAT GAG	CCA GTA	GAT CGG	CCG CCA	GTG CTA	1047
Arg Ser Cys	Lys Ala Lys	Asn Glu	Pro Val	Asp Arg	Pro Pro	Val Leu	
	190		195		200		

FIG. 12A

CTG ATT GCC AAC TCT CAG AAC ATC CTA GCT ACG TAC CTG AGT GGG GCC	1095
Leu Ile Ala Asn Ser Gln Asn Ile Leu Ala Thr Tyr Leu Ser Gly Ala	
205 210 215	
CAA GTG TCT ACC ATC ACA CCC ACC AGC ACC CGA CAA ACC ACG GCC ATG	1143
Gln Val Ser Thr Ile Thr Pro Thr Ser Thr Arg Gln Thr Thr Ala Met	
220 225 230	
GAC TTC AGT TAT GCC AAT GAG ACC GTA TGC TGG GTG CAC GTT GGG GAC	1191
Asp Phe Ser Tyr Ala Asn Glu Thr Val Cys Trp Val His Val Gly Asp	
235 240 245 250	
AGT GCT GCC CAG ACA CAG CTC AAG TGT GCC CGG ATG CCT GGC CTG AAG	1239
Ser Ala Ala Gln Thr Gln Leu Lys Cys Ala Arg Met Pro Gly Leu Lys	
255 260 265	
GGC TTT GTG GAT GAG CAT ACC ATC AAC ATC TCC CTC AGC CTG CAC CAC	1287
Gly Phe Val Asp Glu His Thr Ile Asn Ile Ser Leu Ser Leu His His	
270 275 280	
GTG GAG CAG ATG GCA ATC GAC TGG CTG ACG GGA AAC TTC TAC TTT GTC	1335
Val Glu Gln Met Ala Ile Asp Trp Leu Thr Gly Asn Phe Tyr Phe Val	
285 290 295	
GAC GAC ATT GAC GAC AGG ATC TTT GTC TGT AAC CGA AAC GGG GAC ACC	1383
Asp Asp Ile Asp Asp Arg Ile Phe Val Cys Asn Arg Asn Gly Asp Thr	
300 305 310	
TGT GTC ACT CTG CTG GAC CTG GAA CTC TAC AAC CCC AAA GGC ATC GCC	1431
Cys Val Thr Leu Leu Asp Leu Glu Leu Tyr Asn Pro Lys Gly Ile Ala	
315 320 325 330	
TTG GAC CCC GCC ATG GGG AAG GTG TTC TTC ACT GAC TAC GGG CAG ATC	1479
Leu Asp Pro Ala Met Gly Lys Val Phe Phe Thr Asp Tyr Gly Gln Ile	
335 340 345	
CCA AAG GTG GAG CGC TGT GAC ATG GAT GGA CAG AAC CGC ACC AAG CTG	1527
Pro Lys Val Glu Arg Cys Asp Met Asp Gly Gln Asn Arg Thr Lys Leu	
350 355 360	
GTG GAT AGC AAG ATC GTG TTT CCA CAC GGC ATC ACC CTG GAC CTG GTC	1575
Val Asp Ser Lys Ile Val Phe Pro His Gly Ile Thr Leu Asp Leu Val	
365 370 375	
AGC CGC CTC GTC TAC TGG GCG GAC GCC TAC CTA GAC TAC ATC GAG GTG	1623
Ser Arg Leu Val Tyr Trp Ala Asp Ala Tyr Leu Asp Tyr Ile Glu Val	
380 385 390	
GTA GAC TAC GAA GGG AAG GGT CGG CAG ACC ATC ATC CAA GGC ATC CTG	1671
Val Asp Tyr Glu Gly Lys Gly Arg Gln Thr Ile Ile Gln Gly Ile Leu	
395 400 405 410	
ATC GAG CAC CTG TAC GGC CTG ACC GTG TTT GAG AAC TAT CTC TAC GCC	1719
Ile Glu His Leu Tyr Gly Leu Thr Val Phe Glu Asn Tyr Leu Tyr Ala	
415 420 425	
ACC AAC TCG GAC AAT GCC AAC ACG CAG CAG AAG ACG AGC GTG ATC CGA	1767
Thr Asn Ser Asp Asn Ala Asn Thr Gln Gln Lys Thr Ser Val Ile Arg	
430 435 440	

FIG. 12A

GTG AAC CGG TTC AAC AGT ACT GAG TAC CAG GTC GTC ACC CGT GTG GAC	1815
Val Asn Arg Phe Asn Ser Thr Glu Tyr Gln Val Val Thr Arg Val Asp	
445	450
455	
AAG GGT GGT GCC CTG CAT ATC TAC CAC CAG CGA CGC CAG CCC CGA GTG	1863
Lys Gly Gly Ala Leu His Ile Tyr His Gln Arg Arg Gln Pro Arg Val	
460	465
470	
CGG AGT CAC GCC TGT GAG AAT GAC CAG TAC GGG AAG CCA GGT GGC TGC	1911
Arg Ser His Ala Cys Glu Asn Asp Gln Tyr Gly Lys Pro Gly Gly Cys	
475	480
485	490
TCC GAC ATC TGC CTC CTG GCC AAC AGT CAC AAG GCA AGG ACC TGC AGG	1959
Ser Asp Ile Cys Leu Leu Ala Asn Ser His Lys Ala Arg Thr Cys Arg	
495	500
505	
TGC AGG TCT GGC TTC AGC CTG GGA AGT GAT GGG AAG TCT TGT AAG AAA	2007
Cys Arg Ser Gly Phe Ser Leu Gly Ser Asp Gly Lys Ser Cys Lys Lys	
510	515
520	
CCT GAA CAT GAG CTG TTC CTC GTG TAT GGC AAG GGC CGA CCA GGC ATC	2055
Pro Glu His Glu Leu Phe Leu Val Tyr Gly Lys Gly Arg Pro Gly Ile	
525	530
535	
ATT AGA GGC ATG GAC ATG GGG GCC AAG GTC CCA GAT GAG CAC ATG ATC	2103
Ile Arg Gly Met Asp Met Gly Ala Lys Val Pro Asp Glu His Met Ile	
540	545
550	
CCC ATC GAG AAC CTT ATG AAT CCA CGC GCT CTG GAC TTC CAC GCC GAG	2151
Pro Ile Glu Asn Leu Met Asn Pro Arg Ala Leu Asp Phe His Ala Glu	
555	560
565	570
ACC GGC TTC ATC TAC TTT GCT GAC ACC ACC AGC TAC CTC ATT GGC CGC	2199
Thr Gly Phe Ile Tyr Phe Ala Asp Thr Thr Ser Tyr Leu Ile Gly Arg	
575	580
585	
CAG AAA ATT GAT GGC ACG GAG AGA GAG ACT ATC CTG AAG GAT GGC ATC	2247
Gln Lys Ile Asp Gly Thr Glu Arg Glu Thr Ile Leu Lys Asp Gly Ile	
590	595
600	
CAC AAT GTG GAG GGC GTA GCC GTG GAC TGG ATG GGA GAC AAT CTT TAC	2295
His Asn Val Glu Gly Val Ala Val Asp Trp Met Gly Asp Asn Leu Tyr	
605	610
615	
TGG ACT GAT GAT GGC CCC AAG AAG ACC ATT AGT GTG GCC AGG CTG GAG	2343
Trp Thr Asp Asp Gly Pro Lys Lys Thr Ile Ser Val Ala Arg Leu Glu	
620	625
630	
AAA GCC GCT CAG ACC CGG AAG ACT CTA ATT GAG GGC AAG ATG ACA CAC	2391
Lys Ala Ala Gln Thr Arg Lys Thr Leu Ile Glu Gly Lys Met Thr His	
635	640
645	650
CCC AGG GCC ATT GTA GTG GAT CCA CTC AAT GGG TGG ATG TAC TGG ACA	2439
Pro Arg Ala Ile Val Val Asp Pro Leu Asn Gly Trp Met Tyr Trp Thr	
655	660
665	
GAC TGG GAG GAG GAC CCC AAG GAC AGT CGG CGA GGG CGG CTC GAG AGG	2487
Asp Trp Glu Glu Asp Pro Lys Asp Ser Arg Arg Gly Arg Leu Glu Arg	
670	675
680	

FIG. 12A

GCT Ala	TGG Trp	ATG Met	GAC Asp	GGC Gly	TCA Ser	CAC His	CGA Arg	GAT Asp	ATC Ile	TTT Phe	GTC Val	ACC Thr	TCC Ser	AAG Lys	ACA Thr	2535
	685						690				695					
GTG Val	CTT Leu	TGG Trp	CCC Pro	AAT Asn	GGG Gly	CTA Leu	AGC Ser	CTG Leu	GAT Asp	ATC Ile	CCA Pro	GCC Ala	GGA Gly	CGC Arg	CTC Leu	2583
	700					705					710					
TAC Tyr	TGG Trp	GTG Val	GAT Asp	GCC Ala	TTC Phe	TAT Tyr	GAC Asp	CGA Arg	ATT Ile	GAG Glu	ACC Thr	ATA Ile	CTG Leu	CTC Leu	AAT Asn	2631
715					720					725					730	
GGC Gly	ACA Thr	GAC Asp	CGG Arg	AAG Lys	ATT Ile	GTA Val	TAT Tyr	GAG Glu	GGT Gly	CCT Pro	GAA Glu	CTG Leu	AAT Asn	CAT His	GCC Ala	2679
				735					740						745	
TTC Phe	GGC Gly	CTG Leu	TGT Cys	CAC His	CAT His	GGC Gly	AAC Asn	TAC Tyr	CTC Leu	TTT Phe	TGG Trp	ACC Thr	GAG Glu	TAC Tyr	CGG Arg	2727
			750					755						760		
AGC Ser	GGC Gly	AGC Ser	GTC Val	TAC Tyr	CGC Arg	TTG Leu	GAA Glu	CGG Arg	GGC Gly	GTG Val	GCA Ala	GGC Gly	GCA Ala	CCG Pro	CCC Pro	2775
			765				770					775				
ACT Thr	GTG Val	ACC Thr	CTT Leu	CTG Leu	CGC Arg	AGC Ser	GAG Glu	AGA Arg	CCG Pro	CCT Pro	ATC Ile	TTT Phe	GAG Glu	ATC Ile	CGA Arg	2823
	780					785					790					
ATG Met	TAC Tyr	GAC Asp	GCG Ala	CAC His	GAG Glu	CAG Gln	CAA Gln	GTG Val	GGT Gly	ACC Thr	AAC Asn	AAA Lys	TGC Cys	CGG Arg	GTA Val	2871
795					800					805					810	
AAT Asn	AAC Asn	GGA Gly	GGC Gly	TGC Cys	AGC Ser	AGC Ser	CTG Leu	TGC Cys	CTC Leu	GCC Ala	ACC Thr	CCC Pro	GGG Gly	AGC Ser	CGC Arg	2919
				815					820					825		
CAG Gln	TGT Cys	GCC Ala	TGT Cys	GCC Ala	GAG Glu	GAC Asp	CAG Gln	GTG Val	TTG Leu	GAC Asp	ACA Thr	GAT Asp	GGT Gly	GTC Val	ACC Thr	2967
			830					835					840			
TGC Cys	TTG Leu	GCG Ala	AAC Asn	CCA Pro	TCC Ser	TAC Tyr	GTG Val	CCC Pro	CCA Pro	CCC Pro	CAG Gln	TGC Cys	CAG Gln	CCG Pro	GGC Gly	3015
		845					850					855				
CAG Gln	TTT Phe	GCC Ala	TGT Cys	GCC Ala	AAC Asn	AAC Asn	CGC Arg	TGC Cys	ATC Ile	CAG Gln	GAG Glu	CGC Arg	TGG Trp	AAG Lys	TGT Cys	3063
	860					865					870					
GAC Asp	GGA Gly	GAC Asp	AAC Asn	GAC Asp	TGT Cys	CTG Leu	GAC Asp	AAC Asn	AGC Ser	GAT Asp	GAG Glu	GCC Ala	CCA Pro	GCA Ala	CTG Leu	3111
875					880				885						890	
TGC Cys	CAT His	CAA Gln	CAC His	ACC Thr	TGT Cys	CCC Pro	TCG Ser	GAC Asp	CGA Arg	TTC Phe	AAG Lys	TGT Cys	GAG Glu	AAC Asn	AAC Asn	3159
				895					900					905		
CGG Arg	TGT Cys	ATC Ile	CCC Pro	AAC Asn	CGC Arg	TGG Trp	CTC Leu	TGT Cys	GAT Asp	GGG Gly	GAT Asp	AAT Asn	GAT Asp	TGT Cys	GGC Gly	3207
			910					915						920		

FIG. 12A

AAC AGC GAG GAC GAA TCC AAT GCC ACG TGC TCA GCC CGC ACC TGT CCA Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys Ser Ala Arg Thr Cys Pro 925 930 935	3255
CCC AAC CAG TTC TCC TGT GCC AGT GGC CGA TGC ATT CCT ATC TCA TGG Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg Cys Ile Pro Ile Ser Trp 940 945 950	3303
ACC TGT GAT CTG GAT GAT GAC TGT GGG GAC CGG TCC GAT GAG TCA GCC Thr Cys Asp Leu Asp Asp Asp Cys Gly Asp Arg Ser Asp Glu Ser Ala 955 960 965 970	3351
TCA TGC GCC TAC CCC ACC TGC TTC CCC CTG ACT CAA TTT ACC TGC AAC Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu Thr Gln Phe Thr Cys Asn 975 980 985	3399
AAT GGC AGA TGT ATT AAC ATC AAC TGG CGG TGT GAC AAC GAC AAT GAC Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg Cys Asp Asn Asp Asn Asp 990 995 1000	3447
TGT GGG GAC AAC AGC GAC GAA GCC GGC TGC AGT CAC TCC TGC TCC AGT Cys Gly Asp Asn Ser Asp Glu Ala Gly Cys Ser His Ser Cys Ser Ser 1005 1010 1015	3495
ACC CAG TTC AAG TGC AAC AGT GGC AGA TGC ATC CCC GAG CAC TGG ACG Thr Gln Phe Lys Cys Asn Ser Gly Arg Cys Ile Pro Glu His Trp Thr 1020 1025 1030	3543
TGT GAT GGG GAC AAT GAT TGT GGG GAC TAC AGC GAC GAG ACA CAC GCC Cys Asp Gly Asp Asn Asp Cys Gly Asp Tyr Ser Asp Glu Thr His Ala 1035 1040 1045 1050	3591
AAC TGT ACC AAC CAG GCT ACA AGA CCT CCT GGT GGC TGC CAC TCG GAT Asn Cys Thr Asn Gln Ala Thr Arg Pro Pro Gly Gly Cys His Ser Asp 1055 1060 1065	3639
GAG TTC CAG TGC CCG CTA GAT GGC CTG TGC ATC CCC CTG AGG TGG CGC Glu Phe Gln Cys Pro Leu Asp Gly Leu Cys Ile Pro Leu Arg Trp Arg 1070 1075 1080	3687
TGC GAC GGG GAC ACC GAC TGC ATG GAT TCC AGC GAT GAG AAG AGC TGT Cys Asp Gly Asp Thr Asp Cys Met Asp Ser Ser Asp Glu Lys Ser Cys 1085 1090 1095	3735
GAG GGC GTG ACC CAT GTT TGT GAC CCG AAT GTC AAG TTT GGC TGC AAG Glu Gly Val Thr His Val Cys Asp Pro Asn Val Lys Phe Gly Cys Lys 1100 1105 1110	3783
GAC TCC GCC CGG TGC ATC AGC AAG GCG TGG GTG TGT GAT GGC GAC AGC Asp Ser Ala Arg Cys Ile Ser Lys Ala Trp Val Cys Asp Gly Asp Ser 1115 1120 1125 1130	3831
GAC TGT GAA GAT AAC TCC GAC GAG GAG AAC TGT GAG GCC CTG GCC TGC Asp Cys Glu Asp Asn Ser Asp Glu Glu Asn Cys Glu Ala Leu Ala Cys 1135 1140 1145	3879
AGG CCA CCC TCC CAT CCC TGC GCC AAC AAC ACC TCT GTC TGC CTG CCT Arg Pro Pro Ser His Pro Cys Ala Asn Asn Thr Ser Val Cys Leu Pro 1150 1155 1160	3927

FIG. 12A

CCT GAC AAG CTG TGC GAC GGC AAG GAT GAC TGT GGA GAC GGC TCG GAT	3975
Pro Asp Lys Leu Cys Asp Gly Lys Asp Asp Cys Gly Asp Gly Ser Asp	
1165 1170 1175	
GAG GGC GAG CTC TGT GAC CAG TGT TCT CTG AAT AAT GGT GGC TGT AGT	4023
Glu Gly Glu Leu Cys Asp Gln Cys Ser Leu Asn Asn Gly Gly Cys Ser	
1180 1185 1190	
CAC AAC TGC TCA GTG GCC CCT GGT GAA GGC ATC GTG TGC TCT TGC CCT	4071
His Asn Cys Ser Val Ala Pro Gly Glu Gly Ile Val Cys Ser Cys Pro	
1195 1200 1205 1210	
CTG GGC ATG GAG CTG GGC TCT GAC AAC CAC ACC TGC CAG ATC CAG AGC	4119
Leu Gly Met Glu Leu Gly Ser Asp Asn His Thr Cys Gln Ile Gln Ser	
1215 1220 1225	
TAC TGT GCC AAG CAC CTC AAA TGC AGC CAG AAG TGT GAC CAG AAC AAG	4167
Tyr Cys Ala Lys His Leu Lys Cys Ser Gln Lys Cys Asp Gln Asn Lys	
1230 1235 1240	
TTC AGT GTG AAG TGC TCC TGC TAC GAG GGC TGG GTC TTG GAG CCT GAC	4215
Phe Ser Val Lys Cys Ser Cys Tyr Glu Gly Trp Val Leu Glu Pro Asp	
1245 1250 1255	
GGG GAA ACG TGC CGC AGT CTG GAT CCC TTC AAA CTG TTC ATC ATC TTC	4263
Gly Glu Thr Cys Arg Ser Leu Asp Pro Phe Lys Leu Phe Ile Ile Phe	
1260 1265 1270	
TCC AAC CGC CAC GAG ATC AGG CGC ATT GAC CTT CAC AAG GGG GAC TAC	4311
Ser Asn Arg His Glu Ile Arg Arg Ile Asp Leu His Lys Gly Asp Tyr	
1275 1280 1285 1290	
AGC GTC CTA GTG CCT GGC CTG CGC AAC ACT ATT GCC CTG GAC TTC CAC	4359
Ser Val Leu Val Pro Gly Leu Arg Asn Thr Ile Ala Leu Asp Phe His	
1295 1300 1305	
CTC AGC CAG AGT GCC CTC TAC TGG ACC GAC GCG GTA GAG GAC AAG ATC	4407
Leu Ser Gln Ser Ala Leu Tyr Trp Thr Asp Ala Val Glu Asp Lys Ile	
1310 1315 1320	
TAC CGT GGG AAA CTC CTG GAC AAC GGA GCC CTG ACC AGC TTT GAG GTG	4455
Tyr Arg Gly Lys Leu Leu Asp Asn Gly Ala Leu Thr Ser Phe Glu Val	
1325 1330 1335	
GTG ATT CAG TAT GGC TTG GCC ACA CCA GAG GGC CTG GCT GTA GAT TGG	4503
Val Ile Gln Tyr Gly Leu Ala Thr Pro Glu Gly Leu Ala Val Asp Trp	
1340 1345 1350	
ATT GCA GGC AAC ATC TAC TGG GTG GAG AGC AAC CTG GAC CAG ATC GAA	4551
Ile Ala Gly Asn Ile Tyr Trp Val Glu Ser Asn Leu Asp Gln Ile Glu	
1355 1360 1365 1370	
GTG GCC AAG CTG GAC GGA ACC CTC CGA ACC ACT CTG CTG GCG GGT GAC	4599
Val Ala Lys Leu Asp Gly Thr Leu Arg Thr Thr Leu Leu Ala Gly Asp	
1375 1380 1385	
ATT GAG CAC CCG AGG GCC ATC GCT CTG GAC CCT CGG GAT GGG ATT CTG	4647
Ile Glu His Pro Arg Ala Ile Ala Leu Asp Pro Arg Asp Gly Ile Leu	
1390 1395 1400	

FIG. 12A

TTT TGG ACA GAC TGG GAT GCC AGC CTG CCA CGA ATC GAG GCT GCA TCC Phe Trp Thr Asp Trp Asp Ala Ser Leu Pro Arg Ile Glu Ala Ala Ser 1405 1410 1415	4695
ATG AGT GGA GCT GGC CGC CGA ACC ATC CAC CGG GAG ACA GGC TCT GGG Met Ser Gly Ala Gly Arg Arg Thr Ile His Arg Glu Thr Gly Ser Gly 1420 1425 1430	4743
GGC TGC GCC AAT GGG CTC ACC GTG GAT TAC CTG GAG AAG CGC ATC CTC Gly Cys Ala Asn Gly Leu Thr Val Asp Tyr Leu Glu Lys Arg Ile Leu 1435 1440 1445 1450	4791
TGG ATT GAT GCT AGG TCA GAT GCC ATC TAT TCA GCC CGG TAT GAC GGC Trp Ile Asp Ala Arg Ser Asp Ala Ile Tyr Ser Ala Arg Tyr Asp Gly 1455 1460 1465	4839
TCC GGC CAC ATG GAG GTG CTT CGG GGA CAC GAG TTC CTG TCA CAC CCA Ser Gly His Met Glu Val Leu Arg Gly His Glu Phe Leu Ser His Pro 1470 1475 1480	4887
TTT GCC GTG ACA CTG TAC GGT GGG GAG GTG TAC TGG ACC GAC TGG CGA Phe Ala Val Thr Leu Tyr Gly Gly Glu Val Tyr Trp Thr Asp Trp Arg 1485 1490 1495	4935
ACA AAT ACA CTG GCT AAG GCC AAC AAG TGG ACT GGC CAC AAC GTC ACC Thr Asn Thr Leu Ala Lys Ala Asn Lys Trp Thr Thr Gly His Asn Val Thr 1500 1505 1510	4983
GTG GTA CAG AGG ACC AAC ACC CAG CCC TTC GAC CTG CAG GTG TAT CAC Val Val Gln Arg Thr Asn Thr Gln Pro Phe Asp Leu Gln Val Tyr His 1515 1520 1525 1530	5031
CCT TCC CGG CAG CCC ATG GCT CCA AAC CCA TGT GAG GCC AAT GGC GGC Pro Ser Arg Gln Pro Met Ala Pro Asn Pro Cys Glu Ala Asn Gly Gly 1535 1540 1545	5079
CGG GGC CCC TGT TCC CAT CTG TGC CTC ATC AAC TAC AAC CGG ACC GTC Arg Gly Pro Cys Ser His Leu Cys Leu Ile Asn Tyr Asn Arg Thr Val 1550 1555 1560	5127
TCC TGG GCC TGT CCC CAC CTC ATG AAG CTG CAC AAG GAC AAC ACC ACC Ser Trp Ala Cys Pro His Leu Met Lys Leu His Lys Asp Asn Thr Thr 1565 1570 1575	5175
TGC TAT GAG TTT AAG AAG TTC CTG CTG TAC GCA CGT CAG ATG GAG ATC Cys Tyr Glu Phe Lys Lys Phe Leu Leu Tyr Ala Arg Gln Met Glu Ile 1580 1585 1590	5223
CGG GGC GTG GAC CTG GAT GCC CCG TAC TAC AAT TAT ATC ATC TCC TTC Arg Gly Val Asp Leu Asp Ala Pro Tyr Tyr Asn Tyr Ile Ile Ser Phe 1595 1600 1605 1610	5271
ACG GTG CCT GAT ATC GAC AAT GTC ACG GTG CTG GAC TAT GAT GCC CGA Thr Val Pro Asp Ile Asp Asn Val Thr Val Leu Asp Tyr Asp Ala Arg 1615 1620 1625	5319
GAG CAG CGA GTT TAC TGG TCT GAT GTG CGG ACT CAA GCC ATC AAA AGG Glu Gln Arg Val Tyr Trp Ser Asp Val Arg Thr Gln Ala Ile Lys Arg 1630 1635 1640	5367

FIG. 12A

GCA TTT ATC AAC GGC ACT GGC GTG GAG ACC GTT GTC TCT GCA GAC TTG Ala Phe Ile Asn Gly Thr Gly Val Glu Thr Val Val Ser Ala Asp Leu 1645 1650 1655	5415
CCC AAC GCC CAC GGG CTG GCT GTG GAC TGG GTC TCC CGA AAT CTG TTT Pro Asn Ala His Gly Leu Ala Val Asp Trp Val Ser Arg Asn Leu Phe 1660 1665 1670	5463
TGG ACA AGT TAC GAC ACC AAC AAG AAG CAG ATT AAC GTG GCC CGG CTG Trp Thr Ser Tyr Asp Thr Asn Lys Lys Gln Ile Asn Val Ala Arg Leu 1675 1680 1685 1690	5511
GAC GGC TCC TTC AAG AAT GCG GTG GTG CAG GGC CTG GAG CAG CCC CAC Asp Gly Ser Phe Lys Asn Ala Val Val Gln Gly Leu Glu Gln Pro His 1695 1700 1705	5559
GGC CTG GTC GTC CAC CCG CTT CGT GGC AAG CTC TAC TGG ACT GAT GGG Gly Leu Val Val His Pro Leu Arg Gly Lys Leu Tyr Trp Thr Asp Gly 1710 1715 1720	5607
GAC AAC ATC AGC ATG GCC AAC ATG GAT GGG AGC AAC CAC ACT CTG CTC Asp Asn Ile Ser Met Ala Asn Met Asp Gly Ser Asn His Thr Leu Leu 1725 1730 1735	5655
TTC AGT GGC CAG AAG GGC CCT GTG GGG TTG GCC ATT GAC TTC CCT GAG Phe Ser Gly Gln Lys Gly Pro Val Gly Leu Ala Ile Asp Phe Pro Glu 1740 1745 1750	5703
AGC AAA CTC TAC TGG ATC AGC TCT GGG AAC CAC ACA ATC AAC CGT TGC Ser Lys Leu Tyr Trp Ile Ser Ser Gly Asn His Thr Ile Asn Arg Cys 1755 1760 1765 1770	5751
AAT CTG GAT GGG AGC GAG CTG GAG GTC ATC GAC ACC ATG CGG AGC CAG Asn Leu Asp Gly Ser Glu Leu Glu Val Ile Asp Thr Met Arg Ser Gln 1775 1780 1785	5799
CTG GGC AAG GCC ACT GCC CTG GCC ATC ATG GGG GAC AAG CTG TGG TGG Leu Gly Lys Ala Thr Ala Leu Ala Ile Met Gly Asp Lys Leu Trp Trp 1790 1795 1800	5847
GCA GAT CAG GTG TCA GAG AAG ATG GGC ACG TGC AAC AAA GCC GAT GGC Ala Asp Gln Val Ser Glu Lys Met Gly Thr Cys Asn Lys Ala Asp Gly 1805 1810 1815	5895
TCT GGG TCC GTG GTG CTG CGG AAC AGT ACC ACG TTG GTT ATG CAC ATG Ser Gly Ser Val Val Leu Arg Asn Ser Thr Thr Leu Val Met His Met 1820 1825 1830	5943
AAG GTG TAT GAC GAG AGC ATC CAG CTA GAG CAT GAG GGC ACC AAC CCC Lys Val Tyr Asp Glu Ser Ile Gln Leu Glu His Glu Gly Thr Asn Pro 1835 1840 1845 1850	5991
TGC AGT GTC AAC AAC GGA GAC TGT TCC CAG CTC TGC CTG CCA ACA TCA Cys Ser Val Asn Asn Gly Asp Cys Ser Gln Leu Cys Leu Pro Thr Ser 1855 1860 1865	6039
GAG ACG ACT CGC TCC TGT ATG TGT ACA GCC GGT TAC AGC CTC CGG AGC Glu Thr Thr Arg Ser Cys Met Cys Thr Ala Gly Tyr Ser Leu Arg Ser 1870 1875 1880	6087

FIG. 12A

GGA CAG CAG GCC TGT GAG GGT GTG GGC TCT TTT CTC CTG TAC TCT GTA Gly Gln Gln Ala Cys Glu Gly Val Gly Ser Phe Leu Leu Tyr Ser Val 1885 1890 1895	6135
CAT GAG GGA ATT CGG GGG ATT CCA CTA GAT CCC AAT GAC AAG TCG GAT His Glu Gly Ile Arg Gly Ile Pro Leu Asp Pro Asn Asp Lys Ser Asp 1900 1905 1910	6183
GCC CTG GTC CCA GTG TCC GGA ACT TCA CTG GCT GTC GGA ATC GAC TTC Ala Leu Val Pro Val Ser Gly Thr Ser Leu Ala Val Gly Ile Asp Phe 1915 1920 1925 1930	6231
CAT GCC GAA AAT GAC ACT ATT TAT TGG GTG GAT ATG GGC CTA AGC ACC His Ala Glu Asn Asp Thr Ile Tyr Trp Val Asp Met Gly Leu Ser Thr 1935 1940 1945	6279
ATC AGC AGG GCC AAG CGT GAC CAG ACA TGG CGA GAG GAT GTG GTG ACC Ile Ser Arg Ala Lys Arg Asp Gln Thr Trp Arg Glu Asp Val Val Thr 1950 1955 1960	6327
AAC GGT ATT GGC CGT GTG GAG GGC ATC GCC GTG GAC TGG ATC GCA GGC Asn Gly Ile Gly Arg Val Glu Gly Ile Ala Val Asp Trp Ile Ala Gly 1965 1970 1975	6375
AAC ATA TAC TGG ACG GAC CAG GGC TTC GAT GTC ATC GAG GTT GCC CGG Asn Ile Tyr Trp Thr Asp Gln Gly Phe Asp Val Ile Glu Val Ala Arg 1980 1985 1990	6423
CTC AAT GGC TCT TTT CGT TAT GTG GTC ATT TCC CAG GGT CTG GAC AAG Leu Asn Gly Ser Phe Arg Tyr Val Val Ile Ser Gln Gly Leu Asp Lys 1995 2000 2005 2010	6471
CCT CGG GCC ATC ACT GTC CAC CCA GAG AAG GGG TAC TTG TTC TGG ACC Pro Arg Ala Ile Thr Val His Pro Glu Lys Gly Tyr Leu Phe Trp Thr 2015 2020 2025	6519
GAG TGG GGT CAT TAC CCA CGT ATT GAG CGG TCT CGC CTT GAT GGC ACA Glu Trp Gly His Tyr Pro Arg Ile Glu Arg Ser Arg Leu Asp Gly Thr 2030 2035 2040	6567
GAG AGA GTG GTG TTG GTT AAT GTC AGC ATC AGC TGG CCC AAT GGC ATC Glu Arg Val Val Leu Val Asn Val Ser Ile Ser Trp Pro Asn Gly Ile 2045 2050 2055	6615
TCA GTA GAC TAT CAG GGC GGC AAG CTC TAC TGG TGT GAT GCT CGG ATG Ser Val Asp Tyr Gln Gly Gly Lys Leu Tyr Trp Cys Asp Ala Arg Met 2060 2065 2070	6663
GAC AAG ATC GAG CGC ATC GAC CTG GAA ACG GGC GAG AAC CGG GAG GTG Asp Lys Ile Glu Arg Ile Asp Leu Glu Thr Gly Glu Asn Arg Glu Val 2075 2080 2085 2090	6711
GTC CTG TCC AGC AAT AAC ATG GAT ATG TTC TCC GTG TCC GTG TTT GAG Val Leu Ser Ser Asn Asn Met Asp Met Phe Ser Val Ser Val Phe Glu 2095 2100 2105	6759
GAC TTC ATC TAC TGG AGT GAC AGA ACT CAC GCC AAT GGC TCC ATC AAG Asp Phe Ile Tyr Trp Ser Asp Arg Thr His Ala Asn Gly Ser Ile Lys 2110 2115 2120	6807

FIG. 12A

CGC Arg	GGC Gly	TGC Cys	AAA Lys	GAC Asp	AAT Asn	GCT Ala	ACA Thr	GAC Asp	TCC Ser	GTG Val	CCT Pro	CTG Leu	AGG Arg	ACA Thr	GGC Gly	6855
		2125					2130					2135				
ATT Ile	GGT Gly	GTT Val	CAG Gln	CTT Leu	AAA Lys	GAC Asp	ATC Ile	AAG Lys	GTC Val	TTC Phe	AAC Asn	AGG Arg	GAC Asp	AGG Arg	CAG Gln	6903
	2140					2145					2150					
AAG Lys	GGT Gly	ACC Thr	AAT Asn	GTG Val	TGC Cys	GCG Ala	GTA Val	GCC Ala	AAC Asn	GGC Gly	GGG Gly	TGC Cys	CAG Gln	CAG Gln	CTC Leu	6951
2155					2160					2165					2170	
TGC Cys	TTG Leu	TAT Tyr	CGG Arg	GGT Gly	GGC Gly	GGA Gln	CAG Arg	CGA Ala	GCC Ala	TGT Cys	GCC Ala	TGT Cys	GCC Ala	CAC His	GGG Gly	6999
			2175						2180					2185		
ATG Met	CTG Leu	GCA Ala	GAA Glu	GAC Asp	GGG Gly	GCC Ala	TCA Ser	TGC Cys	CGA Arg	GAG Glu	TAC Tyr	GCT Ala	GGC Gly	TAC Tyr	CTG Leu	7047
			2190						2195					2200		
CTC Leu	TAC Tyr	TCA Ser	GAG Glu	CGG Arg	ACC Thr	ATC Ile	CTC Leu	AAG Lys	AGC Ser	ATC Ile	CAC His	CTG Leu	TCG Ser	GAT Asp	GAG Glu	7095
			2205					2210						2215		
CGT Arg	AAC Asn	CTC Leu	AAC Asn	GCA Ala	CCG Pro	GTG Val	CAG Gln	CCC Pro	TTT Phe	GAA Glu	GAC Asp	CCC Pro	GAG Glu	CAC His	ATG Met	7143
		2220				2225						2230				
AAA Lys	AAT Asn	GTC Val	ATC Ile	GCC Ala	CTG Leu	GCC Ala	TTT Phe	GAC Asp	TAC Tyr	CGA Arg	GCA Ala	GGC Gly	ACC Thr	TCC Ser	CCG Pro	7191
2235					2240					2245					2250	
GGG Gly	ACC Thr	CCT Pro	AAC Asn	CGC Arg	ATC Ile	TTC Phe	TTC Phe	AGT Ser	GAC Asp	ATC Ile	CAC His	TTT Phe	GGG Gly	AAC Asn	ATC Ile	7239
				2255					2260					2265		
CAG Gln	CAG Gln	ATC Ile	AAT Asn	GAC Asp	GAT Asp	GGC Gly	TCG Ser	GGC Gly	AGG Arg	ACC Thr	ACC Thr	ATC Ile	GTG Val	GAA Glu	AAT Asn	7287
			2270						2275					2280		
GTG Val	GGC Gly	TCT Ser	GTG Val	GAA Glu	GGC Gly	CTG Leu	GCC Ala	TAT Tyr	CAC His	CGT Arg	GGC Gly	TGG Trp	GAC Asp	ACA Thr	CTG Leu	7335
		2285					2290							2295		
TAC Tyr	TGG Trp	ACA Thr	AGC Ser	TAC Tyr	ACC Thr	ACA Thr	TCC Ser	ACC Thr	ATC Ile	ACC Thr	CGC Arg	CAC His	ACC Thr	GTG Val	GAC Asp	7383
	2300						2305							2310		
CAG Gln	ACT Thr	CGC Arg	CCA Pro	GGG Gly	GCC Ala	TTC Phe	GAG Glu	AGG Arg	GAG Glu	ACA Thr	GTC Val	ATC Ile	ACC Thr	ATG Met	TCC Ser	7431
2315					2320					2325					2330	
GGA Gly	GAC Asp	GAC Asp	CAC His	CCG Pro	AGA Arg	GCC Ala	TTT Phe	GTG Val	CTG Leu	GAT Asp	GAG Glu	TGC Cys	CAG Gln	AAC Asn	CTG Leu	7479
				2335					2340					2345		
ATG Met	TTC Phe	TGG Trp	ACC Thr	AAT Asn	TGG Trp	AAC Asn	GAG Glu	CTC Leu	CAT His	CCA Pro	AGC Ser	ATC Ile	ATG Met	CGG Arg	GCA Ala	7527
			2350						2355					2360		

FIG. 12A

GCC CTA TCC GGA GCC AAC GTC CTG ACC CTC ATT GAG AAG GAC ATC CGC Ala Leu Ser Gly Ala Asn Val Leu Thr Leu Ile Glu Lys Asp Ile Arg 2365 2370 2375	7575
ACG CCC AAT GGG TTG GCC ATC GAC CAC CGG GCG GAG AAG CTG TAC TTC Thr Pro Asn Gly Leu Ala Ile Asp His Arg Ala Glu Lys Leu Tyr Phe 2380 2385 2390	7623
TCG GAT GCC ACC TTG GAC AAG ATC GAG CGC TGC GAG TAC GAC GGC TCC Ser Asp Ala Thr Leu Asp Lys Ile Glu Arg Cys Glu Tyr Asp Gly Ser 2395 2400 2405 2410	7671
CAC CGC TAT GTG ATC CTA AAG TCG GAG CCC GTC CAC CCC TTT GGG TTG His Arg Tyr Val Ile Leu Lys Ser Glu Pro Val His Pro Phe Gly Leu 2415 2420 2425	7719
GCG GTG TAC GGA GAG CAC ATT TTC TGG ACT GAC TGG GTG CGG CGG GCT Ala Val Tyr Gly Glu His Ile Phe Trp Thr Asp Trp Val Arg Arg Ala 2430 2435 2440	7767
GTG CAG CGA GCC AAC AAG TAT GTG GGC AGC GAC ATG AAG CTG CTT CGG Val Gln Arg Ala Asn Lys Tyr Val Gly Ser Asp Met Lys Leu Leu Arg 2445 2450 2455	7815
GTG GAC ATT CCC CAG CAA CCC ATG GGC ATC ATC GCC GTG GCC AAT GAC Val Asp Ile Pro Gln Gln Pro Met Gly Ile Ile Ala Val Ala Asn Asp 2460 2465 2470	7863
ACC AAC AGC TGT GAA CTC TCC CCC TGC CGT ATC AAC AAT GGA GGC TGC Thr Asn Ser Cys Glu Leu Ser Pro Cys Arg Ile Asn Asn Gly Gly Cys 2475 2480 2485 2490	7911
CAG GAT CTG TGT CTG CTC ACC CAC CAA GGC CAC GTC AAC TGT TCC TGT Gln Asp Leu Cys Leu Leu Thr His Gln Gly His Val Asn Cys Ser Cys 2495 2500 2505	7959
CGA GGG GGC CGG ATC CTC CAG GAG GAC TTC ACC TGC CGG GCT GTG AAC Arg Gly Gly Arg Ile Leu Gln Glu Asp Phe Thr Cys Arg Ala Val Asn 2510 2515 2520	8007
TCC TCT TGT CGG GCA CAA GAT GAG TTT GAG TGT GCC AAT GGG GAA TGT Ser Ser Cys Arg Ala Gln Asp Glu Phe Glu Cys Ala Asn Gly Glu Cys 2525 2530 2535	8055
ATC AGC TTC AGC CTC ACC TGT GAT GGC GTC TCC CAC TGC AAG GAC AAG Ile Ser Phe Ser Leu Thr Cys Asp Gly Val Ser His Cys Lys Asp Lys 2540 2545 2550	8103
TCC GAT GAG AAG CCC TCC TAC TGC AAC TCA CGC CGC TGC AAG AAG ACT Ser Asp Glu Lys Pro Ser Tyr Cys Asn Ser Arg Arg Cys Lys Lys Thr 2555 2560 2565 2570	8151
TTC CGC CAG TGT AAC AAT GGC CGC TGT GTA TCC AAC ATG CTG TGG TGC Phe Arg Gln Cys Asn Asn Gly Arg Cys Val Ser Asn Met Leu Trp Cys 2575 2580 2585	8199
AAT GGG GTG GAT TAC TGT GGG GAT GGC TCT GAT GAG ATA CCT TGC AAC Asn Gly Val Asp Tyr Cys Gly Asp Gly Ser Asp Glu Ile Pro Cys Asn 2590 2595 2600	8247

FIG. 12A

AAG ACT GCC TGT GGT GTG GGT GAG TTC CGC TGC CGG GAT GGG TCC TGC Lys Thr Ala Cys Gly Val Gly Glu Phe Arg Cys Arg Asp Gly Ser Cys 2605 2610 2615	8295
ATC GGG AAC TCC AGT CGC TGC AAC CAG TTT GTG GAT TGT GAG GAT GCC Ile Gly Asn Ser Ser Arg Cys Asn Gln Phe Val Asp Cys Glu Asp Ala 2620 2625 2630	8343
TCG GAT GAG ATG AAT TGC AGT GCC ACA GAC TGC AGC AGC TAT TTC CGC Ser Asp Glu Met Asn Cys Ser Ala Thr Asp Cys Ser Ser Tyr Phe Arg 2635 2640 2645 2650	8391
CTG GGC GTG AAA GGT GTC CTC TTC CAG CCG TGC GAG CGG ACA TCC CTG Leu Gly Val Lys Gly Val Leu Phe Gln Pro Cys Glu Arg Thr Ser Leu 2655 2660 2665	8439
TGC TAC GCA CCT AGC TGG GTG TGT GAT GGC GCC AAC GAC TGT GGA GAC Cys Tyr Ala Pro Ser Trp Val Cys Asp Gly Ala Asn Asp Cys Gly Asp 2670 2675 2680	8487
TAC AGC GAT GAA CGT GAC TGT CCA GGT GTG AAG CGC CCT AGG TGC CCG Tyr Ser Asp Glu Arg Asp Cys Pro Gly Val Lys Arg Pro Arg Cys Pro 2685 2690 2695	8535
CTC AAT TAC TTT GCC TGC CCC AGC GGG CGC TGT ATC CCC ATG AGC TGG Leu Asn Tyr Phe Ala Cys Pro Ser Gly Arg Cys Ile Pro Met Ser Trp 2700 2705 2710	8583
ACG TGT GAC AAG GAG GAT GAC TGT GAG AAC GGC GAG GAT GAG ACC CAC Thr Cys Asp Lys Glu Asp Asp Cys Glu Asn Gly Glu Asp Glu Thr His 2715 2720 2725 2730	8631
TGC AAC AAG TTC TGC TCA GAG GCA CAG TTC GAG TGC CAG AAC CAC CGG Cys Asn Lys Phe Cys Ser Glu Ala Gln Phe Glu Cys Gln Asn His Arg 2735 2740 2745	8679
TGT ATC TCC AAG CAG TGG CTG TGT GAC GGT AGC GAT GAT TGC GGG GAT Cys Ile Ser Lys Gln Trp Leu Cys Asp Gly Ser Asp Asp Cys Gly Asp 2750 2755 2760	8727
GGC TCC GAT GAG GCA GCT CAC TGT GAA GGC AAG ACA TGT GGC CCC TCC Gly Ser Asp Glu Ala Ala His Cys Glu Gly Lys Thr Cys Gly Pro Ser 2765 2770 2775	8775
TCC TTC TCC TGT CCC GGC ACC CAC GTG TGT GTC CCT GAG CGC TGG CTC Ser Phe Ser Cys Pro Gly Thr His Val Cys Val Pro Glu Arg Trp Leu 2780 2785 2790	8823
TGT GAT GGC GAC AAG GAC TGT ACC GAT GGC GCG GAT GAG AGT GTC ACT Cys Asp Gly Asp Lys Asp Cys Thr Asp Gly Ala Asp Glu Ser Val Thr 2795 2800 2805 2810	8871
GCT GGC TGC CTG TAC AAC AGC ACC TGT GAT GAC CGT GAG TTC ATG TGC Ala Gly Cys Leu Tyr Asn Ser Thr Cys Asp Asp Arg Glu Phe Met Cys 2815 2820 2825	8919
CAG AAC CGC TTG TGT ATT CCC AAG CAT TTC GTG TGC GAC CAT GAC CGT Gln Asn Arg Leu Cys Ile Pro Lys His Phe Val Cys Asp His Asp Arg 2830 2835 2840	8967

FIG. 12A

GAC TGT GCT GAT GGC TCT GAT GAA TCC CCT GAG TGT GAG TAC CCA ACC Asp Cys Ala Asp Gly Ser Asp Glu Ser Pro Glu Cys Glu Tyr Pro Thr 2845 2850 2855	9015
TGC GGG CCC AAT GAA TTC CGC TGT GCC AAT GGG CGT TGT CTG AGC TCC Cys Gly Pro Asn Glu Phe Arg Cys Ala Asn Gly Arg Cys Leu Ser Ser 2860 2865 2870	9063
CGT CAG TGG GAA TGT GAT GGG GAG AAT GAC TGT CAC GAC CAC AGC GAT Arg Gln Trp Glu Cys Asp Gly Glu Asn Asp Cys His Asp His Ser Asp 2875 2880 2885 2890	9111
GAG GCT CCC AAG AAC CCA CAC TGC ACC AGC CCA GAG CAC AAA TGC AAT Glu Ala Pro Lys Asn Pro His Cys Thr Ser Pro Glu His Lys Cys Asn 2895 2900 2905	9159
GCC TCA TCA CAG TTC CTG TGC AGC AGC GGG CGC TGC GTG GCT GAG GCG Ala Ser Ser Gln Phe Leu Cys Ser Ser Gly Arg Cys Val Ala Glu Ala 2910 2915 2920	9207
TTG CTC TGC AAC GGC CAG GAC GAC TGT GGG GAC GGT TCA GAC GAA CGC Leu Leu Cys Asn Gly Gln Asp Asp Cys Gly Asp Gly Ser Asp Glu Arg 2925 2930 2935	9255
GGG TGC CAT GTC AAC GAG TGT CTC AGC CGC AAG CTC AGT GGC TGC AGT Gly Cys His Val Asn Glu Cys Leu Ser Arg Lys Leu Ser Gly Cys Ser 2940 2945 2950	9303
CAG GAC TGC GAG GAC CTC AAG ATA GGC TTT AAG TGC CGC TGT CGC CCG Gln Asp Cys Glu Asp Leu Lys Ile Gly Phe Lys Cys Arg Cys Arg Pro 2955 2960 2965 2970	9351
GGC TTC CGG CTA AAG GAC GAT GGC AGG ACC TGT GCC GAC CTG GAT GAG Gly Phe Arg Leu Lys Asp Asp Gly Arg Thr Cys Ala Asp Leu Asp Glu 2975 2980 2985	9399
TGC AGC ACC ACC TTC CCC TGC AGC CAG CTC TGC ATC AAC ACC CAC GGA Cys Ser Thr Thr Phe Pro Cys Ser Gln Leu Cys Ile Asn Thr His Gly 2990 2995 3000	9447
AGT TAC AAG TGT CTG TGT GTG GAG GGC TAT GCA CCC CGT GGC GGT GAC Ser Tyr Lys Cys Leu Cys Val Glu Gly Tyr Ala Pro Arg Gly Gly Asp 3005 3010 3015	9495
CCC CAC AGC TGC AAA GCT GTG ACC GAT GAG GAG CCA TTT CTC ATC TTT Pro His Ser Cys Lys Ala Val Thr Asp Glu Glu Pro Phe Leu Ile Phe 3020 3025 3030	9543
GCC AAC CGG TAC TAC CTG CGG AAG CTC AAC CTG GAC GGC TCC AAC TAC Ala Asn Arg Tyr Tyr Leu Arg Lys Leu Asn Leu Asp Gly Ser Asn Tyr 3035 3040 3045 3050	9591
ACA CTG CTT AAG CAG GGC CTG AAC AAT GCG GTC GCC TTG GCA TTT GAC Thr Leu Leu Lys Gln Gly Leu Asn Asn Ala Val Ala Leu Ala Phe Asp 3055 3060 3065	9639
TAC CGA GAG CAG ATG ATC TAC TGG ACG GGC GTG ACC ACC CAG GGC AGC Tyr Arg Glu Gln Met Ile Tyr Trp Thr Gly Val Thr Thr Gln Gly Ser 3070 3075 3080	9687

FIG. 12A

ATG ATT CGC AGG ATG CAC CTC AAC GGC AGC AAC GTG CAG GTT CTG CAC Met Ile Arg Arg Met His Leu Asn Gly Ser Asn Val Gln Val Leu His 3085 3090 3095	9735
CGG ACG GGC CTT AGT AAC CCA GAT GGG CTC GCT GTG GAC TGG GTG GGT Arg Thr Gly Leu Ser Asn Pro Asp Gly Leu Ala Val Asp Trp Val Gly 3100 3105 3110	9783
GGC AAC CTG TAC TGG TGT GAC AAG GGC AGA GAT ACC ATT GAG GTG TCC Gly Asn Leu Tyr Trp Cys Asp Lys Gly Arg Asp Thr Ile Glu Val Ser 3115 3120 3125 3130	9831
AAG CTT AAC GGG GCC TAT CGG ACA GTG CTG GTC AGC TCT GGC CTC CGG Lys Leu Asn Gly Ala Tyr Arg Thr Val Leu Val Ser Ser Gly Leu Arg 3135 3140 3145	9879
GAG CCC AGA GCT CTG GTA GTG GAT GTA CAG AAT GGG TAC CTG TAC TGG Glu Pro Arg Ala Leu Val Val Asp Val Gln Asn Gly Tyr Leu Tyr Trp 3150 3155 3160	9927
ACA GAC TGG GGT GAC CAC TCA CTG ATC GGC CGG ATT GGC ATG GAT GGA Thr Asp Trp Gly Asp His Ser Leu Ile Gly Arg Ile Gly Met Asp Gly 3165 3170 3175	9975
TCT GGC CGC AGC ATC ATC GTG GAC ACT AAG ATC ACA TGG CCC AAT GGC Ser Gly Arg Ser Ile Ile Val Asp Thr Lys Ile Thr Trp Pro Asn Gly 3180 3185 3190	10023
CTG ACC GTG GAC TAC GTC ACG GAA CGC ATC TAC TGG GCT GAC GCC CGT Leu Thr Val Asp Tyr Val Thr Glu Arg Ile Tyr Trp Ala Asp Ala Arg 3195 3200 3205 3210	10071
GAG GAC TAC ATC GAG TTC GCC AGC CTG GAT GGC TCC AAC CGT CAC GTT Glu Asp Tyr Ile Glu Phe Ala Ser Leu Asp Gly Ser Asn Arg His Val 3215 3220 3225	10119
GTG CTG AGC CAA GAC ATC CCA CAC ATC TTT GCG CTG ACC CTA TTT GAA Val Leu Ser Gln Asp Ile Pro His Ile Phe Ala Leu Thr Leu Phe Glu 3230 3235 3240	10167
GAC TAC GTC TAC TGG ACA GAC TGG GAA ACG AAG TCC ATC AAC CGG GCC Asp Tyr Val Tyr Trp Thr Asp Trp Glu Thr Lys Ser Ile Asn Arg Ala 3245 3250 3255	10215
CAC AAG ACC ACG GGT GCC AAC AAA ACA CTC CTC ATC AGC ACC CTG CAC His Lys Thr Thr Gly Ala Asn Lys Thr Leu Leu Ile Ser Thr Leu His 3260 3265 3270	10263
CGG CCC ATG GAC TTA CAT GTA TTC CAC GCC CTG CGC CAG CCA GAT GTG Arg Pro Met Asp Leu His Val Phe His Ala Leu Arg Gln Pro Asp Val 3275 3280 3285 3290	10311
CCC AAT CAC CCC TGC AAA GTC AAC AAT GGT GGC TGC AGC AAC CTG TGC Pro Asn His Pro Cys Lys Val Asn Asn Gly Gly Cys Ser Asn Leu Cys 3295 3300 3305	10359
CTG CTG TCC CCT GGG GGT GGT CAC AAG TGC GCC TGC CCC ACC AAC TTC Leu Leu Ser Pro Gly Gly Gly His Lys Cys Ala Cys Pro Thr Asn Phe 3310 3315 3320	10407

FIG. 12A

TAT CTG GGT GGC GAT GGC CGT ACC TGT GTG TCC AAC TGC ACA GCA AGC Tyr Leu Gly Gly Asp Gly Arg Thr Cys Val Ser Asn Cys Thr Ala Ser 3325 3330 3335	10455
CAG TTT GTG TGC AAA AAT GAC AAG TGC ATC CCC TTC TGG TGG AAG TGT Gln Phe Val Cys Lys Asn Asp Lys Cys Ile Pro Phe Trp Trp Lys Cys 3340 3345 3350	10503
GAC ACG GAG GAC GAC TGT GGG GAT CAC TCA GAC GAG CCT CCA GAC TGT Asp Thr Glu Asp Asp Cys Gly Asp His Ser Asp Glu Pro Pro Asp Cys 3355 3360 3365 3370	10551
CCC GAG TTC AAG TGC CGC CCA GGC CAG TTC CAG TGC TCC ACC GGC ATC Pro Glu Phe Lys Cys Arg Pro Gly Gln Phe Gln Cys Ser Thr Gly Ile 3375 3380 3385	10599
TGC ACC AAC CCT GCC TTC ATC TGT GAT GGG GAC AAT GAC TGC CAA GAC Cys Thr Asn Pro Ala Phe Ile Cys Asp Gly Asp Asn Asp Cys Gln Asp 3390 3395 3400	10647
AAT AGT GAC GAG GCC AAT TGC GAC ATT CAC GTC TGC TTG CCC AGC CAA Asn Ser Asp Glu Ala Asn Cys Asp Ile His Val Cys Leu Pro Ser Gln 3405 3410 3415	10695
TTC AAG TGC ACC AAC ACC AAC CGC TGC ATT CCT GGC ATC TTC CGT TGC Phe Lys Cys Thr Asn Thr Asn Arg Cys Ile Pro Gly Ile Phe Arg Cys 3420 3425 3430	10743
AAT GGG CAG GAC AAC TGC GGG GAC GGC GAG GAT GAG CGG GAT TGC CCT Asn Gly Gln Asp Asn Cys Gly Asp Gly Glu Asp Glu Arg Asp Cys Pro 3435 3440 3445 3450	10791
GAG GTG ACC TGC GCC CCC AAC CAG TTC CAG TGC TCC ATC ACC AAG CGC Glu Val Thr Cys Ala Pro Asn Gln Phe Gln Cys Ser Ile Thr Lys Arg 3455 3460 3465	10839
TGC ATC CCT CGC GTC TGG GTC TGT GAC AGG GAT AAT CAC TGT GTG GAC Cys Ile Pro Arg Val Trp Val Cys Asp Arg Asp Asn His Cys Val Asp 3470 3475 3480	10887
GGC AGT GAT GAG CCT GCC AAC TGT ACC CAA ATG ACC TGT GGA GTG GAT Gly Ser Asp Glu Pro Ala Asn Cys Thr Gln Met Thr Cys Gly Val Asp 3485 3490 3495	10935
GAG TTC CGC TGC AAG GAT TCT GGC CGC TGC ATC CCC GCG CGC TGG AAG Glu Phe Arg Cys Lys Asp Ser Gly Arg Cys Ile Pro Ala Arg Trp Lys 3500 3505 3510	10983
TGT GAC GGA GAA GAT GAC TGT GGG GAT GGT TCA GAT GAG CCC AAG GAA Cys Asp Gly Glu Asp Asp Cys Gly Asp Gly Ser Asp Glu Pro Lys Glu 3515 3520 3525 3530	11031
GAG TGT GAT GAG CGC ACC TGT GAG CCA TAC CAG TTC CGC TGC AAA AAC Glu Cys Asp Glu Arg Thr Cys Glu Pro Tyr Gln Phe Arg Cys Lys Asn 3535 3540 3545	11079
AAC CGC TGT GTC CCA GGC CGT TGG CAA TGT GAC TAC GAC AAC GAC TGC Asn Arg Cys Val Pro Gly Arg Trp Gln Cys Asp Tyr Asp Asn Asp Cys 3550 3555 3560	11127

FIG. 12A

GGA GAT AAC TCG GAC GAG GAG AGC TGC ACA CCT CGG CCC TGC TCT GAG Gly Asp Asn Ser Asp Glu Glu Ser Cys Thr Pro Arg Pro Cys Ser Glu 3565 3570 3575	11175
AGT GAG TTT TTC TGT GCC AAT GGC CGC TGC ATC GCT GGG CGC TGG AAG Ser Glu Phe Phe Cys Ala Asn Gly Arg Cys Ile Ala Gly Arg Trp Lys 3580 3585 3590	11223
TGT GAT GGG GAC CAT GAC TGT GCC GAC GGC TCA GAC GAG AAA GAC TGC Cys Asp Gly Asp His Asp Cys Ala Asp Gly Ser Asp Glu Lys Asp Cys 3595 3600 3610	11271
ACC CCC CGC TGT GAT ATG GAC CAG TTC CAG TGC AAG AGT GGC CAC TGC Thr Pro Arg Cys Asp Met Asp Gln Phe Gln Cys Lys Ser Gly His Cys 3615 3620 3625	11319
ATC CCC CTG CGC TGG CCG TGT GAC GCG GAT GCT GAC TGT ATG GAC GGC Ile Pro Leu Arg Trp Pro Cys Asp Ala Asp Ala Asp Cys Met Asp Gly 3630 3635 3640	11367
AGT GAC GAG GAA GCC TGT GGC ACT GGG GTG AGG ACC TGC CCA TTG GAT Ser Asp Glu Glu Ala Cys Gly Thr Gly Val Arg Thr Cys Pro Leu Asp 3645 3650 3655	11415
GAG TTT CAA TGT AAC AAC ACC TTG TGC AAG CCG CTG GCC TGG AAG TGT Glu Phe Gln Cys Asn Asn Thr Leu Cys Lys Pro Leu Ala Trp Lys Cys 3660 3665 3670	11463
GAT GGA GAG GAC GAC TGT GGG GAC AAC TCA GAT GAG AAC CCC GAG GAA Asp Gly Glu Asp Asp Cys Gly Asp Asn Ser Asp Glu Asn Pro Glu Glu 3675 3680 3685 3690	11511
TGC GCC CGG TTC ATC TGC CCT CCC AAC CGG CCT TTC CGC TGC AAG AAT Cys Ala Arg Phe Ile Cys Pro Pro Asn Arg Pro Phe Arg Cys Lys Asn 3695 3700 3705	11559
GAC CGA GTC TGC CTG TGG ATT GGG CGC CAG TGT GAT GGC GTG GAC AAC Asp Arg Val Cys Leu Trp Ile Gly Arg Gln Cys Asp Gly Val Asp Asn 3710 3715 3720	11607
TGT GGA GAT GGG ACT GAC GAG GAG GAC TGT GAG CCC CCC ACG GCC CAG Cys Gly Asp Gly Thr Asp Glu Glu Asp Cys Glu Pro Pro Thr Ala Gln 3725 3730 3735	11655
AAC CCC CAC TGC AAA GAC AAG AAG GAG TTC CTG TGC CGA AAC CAG CGC Asn Pro His Cys Lys Asp Lys Lys Glu Phe Leu Cys Arg Asn Gln Arg 3740 3745 3750	11703
TGT CTA TCA TCC TCC CTG CGC TGT AAC ATG TTC GAT GAC TGC GGC GAT Cys Leu Ser Ser Ser Leu Arg Cys Asn Met Phe Asp Asp Cys Gly Asp 3755 3760 3765 3770	11751
GGC TCC GAT GAA GAA GAT TGC AGC ATC GAC CCC AAG CTG ACC AGC TGT Gly Ser Asp Glu Glu Asp Cys Ser Ile Asp Pro Lys Leu Thr Ser Cys 3775 3780 3785	11799
GCC ACC AAT GCC AGC ATG TGT GGG GAC GAA GCT CGT TGT GTG CGC ACT Ala Thr Asn Ala Ser Met Cys Gly Asp Glu Ala Arg Cys Val Arg Thr 3790 3795 3800	11847

FIG. 12A

GAG AAA GCT GCC TAC TGT GCC TGC CGC TCG GGC TTC CAT ACT GTG CCG Glu Lys Ala Ala Tyr Cys Ala Cys Arg Ser Gly Phe His Thr Val Pro 3805 3810 3815	11895
GGC CAG CCC GGA TGC CAG GAC ATC AAC GAG TGC CTG CGC TTT GGT ACC Gly Gln Pro Gly Cys Gln Asp Ile Asn Glu Cys Leu Arg Phe Gly Thr 3820 3825 3830	11943
TGC TCT CAG CTC TGG AAC AAA CCC AAG GGA GGC CAC CTC TGC AGC TGT Cys Ser Gln Leu Trp Asn Lys Pro Lys Gly Gly His Leu Cys Ser Cys 3835 3840 3845 3850	11991
GCC CGC AAC TTC ATG AAG ACA CAC AAC ACC TGC AAA GCT GAA GGC TCC Ala Arg Asn Phe Met Lys Thr His Asn Thr Cys Lys Ala Glu Gly Ser 3855 3860 3865	12039
GAG TAC CAG GTG CTA TAC ATC GCG GAT GAC AAC GAG ATC CGC AGC TTG Glu Tyr Gln Val Leu Tyr Ile Ala Asp Asp Asn Glu Ile Arg Ser Leu 3870 3875 3880	12087
TTC CCG GGC CAC CCC CAC TCA GCC TAC GAG CAG ACA TTC CAG GGC GAT Phe Pro Gly His Pro His Ser Ala Tyr Glu Gln Thr Phe Gln Gly Asp 3885 3890 3895	12135
GAG AGT GTC CGC ATA GAT GCC ATG GAT GTC CAT GTC AAG GCC GGC CGT Glu Ser Val Arg Ile Asp Ala Met Asp Val His Val Lys Ala Gly Arg 3900 3905 3910	12183
GTC TAC TGG ACT AAC TGG CAC ACG GGC ACA ATC TCC TAC AGG AGC CTG Val Tyr Trp Thr Asn Trp His Thr Gly Thr Ile Ser Tyr Arg Ser Leu 3915 3920 3925 3930	12231
CCC CCT GCC GCC CCT CCT ACC ACT TCC AAC CGC CAC CGG AGG CAG ATC Pro Pro Ala Ala Pro Pro Thr Thr Ser Asn Arg His Arg Arg Gln Ile 3935 3940 3945	12279
GAC CGG GGT GTC ACC CAC CTC AAT ATT TCA GGG CTG AAG ATG CCG AGG Asp Arg Gly Val Thr His Leu Asn Ile Ser Gly Leu Lys Met Pro Arg 3950 3955 3960	12327
GGT ATC GCT ATC GAC TGG GTG GCC GGG AAT GTG TAC TGG ACC GAT TCC Gly Ile Ala Ile Asp Trp Val Ala Gly Asn Val Tyr Trp Thr Asp Ser 3965 3970 3975	12375
GGC CGA GAC GTG ATT GAG GTG GCG CAA ATG AAG GGC GAG AAC CGC AAG Gly Arg Asp Val Ile Glu Val Ala Gln Met Lys Gly Glu Asn Arg Lys 3980 3985 3990	12423
ACG CTC ATC TCG GGC ATG ATT GAT GAG CCC CAT GCC ATC GTG GTG GAC Thr Leu Ile Ser Gly Met Ile Asp Glu Pro His Ala Ile Val Val Asp 3995 4000 4005 4010	12471
CCT CTG AGG GGC ACC ATG TAC TGG TCA GAC TGG GGG AAC CAC CCC AAG Pro Leu Arg Gly Thr Met Tyr Trp Ser Asp Trp Gly Asn His Pro Lys 4015 4020 4025	12519
ATT GAA ACA GCA GCG ATG GAT GGC ACC CTT CGG GAG ACT CTC GTG CAA Ile Glu Thr Ala Ala Met Asp Gly Thr Leu Arg Glu Thr Leu Val Gln 4030 4035 4040	12567

FIG. 12A

GAC AAC ATT CAG TGG CCT ACA GGG CTG GCT GTG GAC TAT CAC AAT GAA Asp Asn Ile Gln Trp Pro Thr Gly Leu Ala Val Asp Tyr His Asn Glu 4045 4050 4055	12615
CGG CTC TAC TGG GCA GAT GCC AAG CTT TCG GTC ATC GGC AGC ATC CGG Arg Leu Tyr Trp Ala Asp Ala Lys Leu Ser Val Ile Gly Ser Ile Arg 4060 4065 4070	12663
CTC AAC GGC ACT GAC CCC ATT GTG GCT GCT GAC AGC AAA CGA GGC CTA Leu Asn Gly Thr Asp Pro Ile Val Ala Ala Asp Ser Lys Arg Gly Leu 4075 4080 4085 4090	12711
AGT CAC CCC TTC AGC ATC GAT GTG TTT GAA GAC TAC ATC TAC GGA GTC Ser His Pro Phe Ser Ile Asp Val Phe Glu Asp Tyr Ile Tyr Gly Val 4095 4100 4105	12759
ACT TAC ATC AAT AAT CGT GTC TTC AAG ATC CAC AAG TTT GGA CAC AGC Thr Tyr Ile Asn Asn Arg Val Phe Lys Ile His Lys Phe Gly His Ser 4110 4115 4120	12807
CCC TTG TAC AAC CTA ACT GGG GGC CTG AGC CAT GCC TCT GAT GTA GTC Pro Leu Tyr Asn Leu Thr Gly Gly Leu Ser His Ala Ser Asp Val Val 4125 4130 4135	12855
CTT TAC CAT CAA CAC AAG CAG CCT GAA GTG ACC AAC CCC TGT GAC CGC Leu Tyr His Gln His Lys Gln Pro Glu Val Thr Asn Pro Cys Asp Arg 4140 4145 4150	12903
AAG AAA TGC GAA TGG CTG TGT CTG CTG AGC CCC AGC GGG CCT GTC TGC Lys Lys Cys Glu Trp Leu Cys Leu Leu Ser Pro Ser Gly Pro Val Cys 4155 4160 4165 4170	12951
ACC TGT CCC AAT GGA AAG AGG CTG GAT AAT GGC ACC TGT GTG CCT GTG Thr Cys Pro Asn Gly Lys Arg Leu Asp Asn Gly Thr Cys Val Pro Val 4175 4180 4185	12999
CCC TCT CCA ACA CCC CCT CCA GAT GCC CCT AGG CCT GGA ACC TGC ACT Pro Ser Pro Thr Pro Pro Pro Asp Ala Pro Arg Pro Gly Thr Cys Thr 4190 4195 4200	13047
CTG CAG TGC TTC AAT GGT GGT AGT TGT TTC CTC AAC GCT CGG AGG CAG Leu Gln Cys Phe Asn Gly Gly Ser Cys Phe Leu Asn Ala Arg Arg Gln 4205 4210 4215	13095
CCC AAG TGC CGT TGC CAG CCC CGT TAC ACA GGC GAT AAG TGT GAG CTG Pro Lys Cys Arg Cys Gln Pro Arg Tyr Thr Gly Asp Lys Cys Glu Leu 4220 4225 4230	13143
GAT CAG TGC TGG GAA TAC TGT CAC AAC GGA GGC ACC TGT GCG GCT TCC Asp Gln Cys Trp Glu Tyr Cys His Asn Gly Gly Thr Cys Ala Ala Ser 4235 4240 4245 4250	13191
CCA TCT GGC ATG CCC ACG TGC CGC TGT CCC ACT GGC TTC ACG GGC CCC Pro Ser Gly Met Pro Thr Cys Arg Cys Pro Thr Gly Phe Thr Gly Pro 4255 4260 4265	13239
AAA TGC ACC GCA CAG GTG TGT GCA GGC TAC TGC TCT AAC AAC AGC ACC Lys Cys Thr Ala Gln Val Cys Ala Gly Tyr Cys Ser Asn Asn Ser Thr 4270 4275 4280	13287

FIG. 12A

TGC ACC GTC AAC CAG GGC AAC CAG CCC CAG TGC CGA TGT CTA CCT GGC Cys Thr Val Asn Gln Gly Asn Gln Pro Gln Cys Arg Cys Leu Pro Gly 4285 4290 4295	13335
TTC CTG GGC GAC CGT TGC CAG TAC CGG CAG TGC TCT GGC TTC TGT GAG Phe Leu Gly Asp Arg Cys Gln Tyr Arg Gln Cys Ser Gly Phe Cys Glu 4300 4305 4310	13383
AAC TTT GGC ACC TGT CAG ATG GCT GCT GAT GGC TCC CGA CAA TGT CGC Asn Phe Gly Thr Cys Gln Met Ala Ala Asp Gly Ser Arg Gln Cys Arg 4315 4320 4325 4330	13431
TGC ACC GTC TAC TTT GAG GGA CCA AGG TGT GAG GTG AAC AAG TGT AGT Cys Thr Val Tyr Phe Glu Gly Pro Arg Cys Glu Val Asn Lys Cys Ser 4335 4340 4345	13479
CGC TGT CTC CAA GGC GCC TGT GTG GTC AAT AAG CAG ACC GGA GAT GTC Arg Cys Leu Gln Gly Ala Cys Val Val Asn Lys Gln Thr Gly Asp Val 4350 4355 4360	13527
ACA TGC AAC TGC ACT GAT GGC CGG GTA GCC CCC AGT TGT CTC ACC TGC Thr Cys Asn Cys Thr Asp Gly Arg Val Ala Pro Ser Cys Leu Thr Cys 4365 4370 4375	13575
ATC GAT CAC TGT AGC AAT GGT GGC TCC TGC ACC ATG AAC AGC AAG ATG Ile Asp His Cys Ser Asn Gly Gly Ser Cys Thr Met Asn Ser Lys Met 4380 4385 4390	13623
ATG CCT GAG TGC CAG TGC CCG CCC CAT ATG ACA GGA CCC CGG TGC CAG Met Pro Glu Cys Gln Cys Pro Pro His Met Thr Gly Pro Arg Cys Gln 4395 4400 4405 4410	13671
GAG CAG GTT GTT AGT CAG CAA CAG CCT GGG CAT ATG GCC TCC ATC CTG Glu Gln Val Val Ser Gln Gln Gln Pro Gly His Met Ala Ser Ile Leu 4415 4420 4425	13719
ATC CCT CTG CTG CTG CTT CTC CTG CTG CTT CTG GTG GCT GGC GTG GTG Ile Pro Leu Leu Leu Leu Leu Leu Leu Leu Val Ala Gly Val Val 4430 4435 4440	13767
TTC TGG TAT AAG CGG CGA GTC CGA GGG GCT AAG GGC TTC CAG CAC CAG Phe Trp Tyr Lys Arg Arg Val Arg Gly Ala Lys Gly Phe Gln His Gln 4445 4450 4455	13815
CGG ATG ACC AAT GGG GCC ATG AAT GTG GAA ATT GGA AAC CCT ACC TAC Arg Met Thr Asn Gly Ala Met Asn Val Glu Ile Gly Asn Pro Thr Tyr 4460 4465 4470	13863
AAG ATG TAT GAA GGT GGA GAG CCC GAT GAT GTC GGG GGC CTA CTG GAT Lys Met Tyr Glu Gly Gly Glu Pro Asp Asp Val Gly Gly Leu Leu Asp 4475 4480 4485 4490	13911
GCT GAT TTT GCC CTT GAC CCT GAC AAG CCT ACC AAC TTC ACC AAC CCA Ala Asp Phe Ala Leu Asp Pro Asp Lys Pro Thr Asn Phe Thr Asn Pro 4495 4500 4505	13959
GTG TAT GCC ACG CTC TAC ATG GGG GGC CAC GGC AGC CGC CAT TCC CTG Val Tyr Ala Thr Leu Tyr Met Gly Gly His Gly Ser Arg His Ser Leu 4510 4515 4520	14007

FIG. 12A

GCC AGC ACG GAC GAG AAG CGA GAA CTG CTG GGC CGG GGA CCT GAA GAC 14055  
 Ala Ser Thr Asp Glu Lys Arg Glu Leu Leu Gly Arg Gly Pro Glu Asp  
 4525 4530 4535

GAG ATA GGA GAT CCC TTG GCA TAGGGCCCTG CCCCACGGA TGTCCCCAGA AAGC 14110  
 CCCCTGCCAC ATGAGTCTTT CAATGAACCC CCTCCCCAGC CGGCCCTTCT CCGGCCCTGC 14170  
 Glu Ile Gly Asp Pro Leu Ala  
 4540 4545

CGGGTGTACA AATGTAAAA TGAAGGAATT ACTTTTTATA TGTGAGCGAG CAAGCGAGCA 14230  
 AGCACAGTAT TATCTCTTG CATTTCCTTC CTGCCTGCTC CTCAGTATCC CCCCCATGCT 14290  
 GCCTTGAGGG GCGGGGGAGG GCTTTGTGGC TCAAAGGTAT GAAGGAGTCC ACATGTTCCC 14350  
 TACCGAGCAT ACCCCTGGAA GCCTGGCGGC ACGGCCTCCC CACCACGCCT GTGCAAGACA 14410  
 CTC AACGGG CTCCGTGTCC CAGCTTTCCT TTCCTTGGCT CTCTGGGGTT AGTTCAGGGG 14470  
 AGGTGGAGTC CTCTGCTGAC CCTGTCTGGA AGATTTGGCT CTAGCTGAGG AAGGAGTCTT 14530  
 TTAGTTGAGG GAAGTCACCC CAAACCCAG CTCCCACTTT CAGGGGCACC TCTCAGATGG 14590  
 CCATGCTCAG TATCCCTTCC AGACAGGCC TCCCCTCTCT AGCGCCCCCT CTGTGGCTCC 14650  
 TAGGGCTGAA CACATTCTTT GGTAAGTGT CCCCAGCCT CCCATCCCC TGAGGGCCAG 14710  
 GAAGAGTCGG GGCACACCAA GGAAGGGCAA GCGGGCAGCC CCATTTTGGG GACGTGAACG 14770  
 TTTAATAAT TTTGCTGAA TTCCTTTACA ACTAAATAAC ACAGATATTG TTATAAATAA 14830  
 AATTGTAAAA AAAAAAAAA

FIG. 12A

Met Leu Thr Pro Pro Leu Leu Leu Leu Val Pro Leu Leu Ser Ala Leu  
 1 5 10 15  
 Val Ser Gly Ala Thr Met Asp Ala Pro Lys Thr Cys Ser Pro Lys Gln  
 20 25 30  
 Phe Ala Cys Arg Asp Gln Ile Thr Cys Ile Ser Lys Gly Trp Arg Cys  
 35 40 45  
 Asp Gly Glu Arg Asp Cys Pro Asp Gly Ser Asp Glu Ala Pro Glu Ile  
 50 55 60  
 Cys Pro Gln Ser Lys Ala Gln Arg Cys Pro Pro Asn Glu His Ser Cys  
 65 70 75 80  
 Leu Gly Thr Glu Leu Cys Val Pro Met Ser Arg Leu Cys Asn Gly Ile  
 85 90 95  
 Gln Asp Cys Met Asp Gly Ser Asp Glu Gly Ala His Cys Arg Glu Leu  
 100 105 110  
 Arg Ala Asn Cys Ser Arg Met Gly Cys Gln His His Cys Val Pro Thr  
 115 120 125  
 Pro Ser Gly Pro Thr Cys Tyr Cys Asn Ser Ser Phe Gln Leu Glu Ala  
 130 135 140  
 Asp Gly Lys Thr Cys Lys Asp Phe Asp Glu Cys Ser Val Tyr Gly Thr  
 145 150 155 160  
 Cys Ser Gln Leu Cys Thr Asn Thr Asp Gly Ser Phe Thr Cys Gly Cys  
 165 170 175  
 Val Glu Gly Tyr Leu Leu Gln Pro Asp Asn Arg Ser Cys Lys Ala Lys  
 180 185 190  
 Asn Glu Pro Val Asp Arg Pro Pro Val Leu Leu Ile Ala Asn Ser Gln  
 195 200 205  
 Asn Ile Leu Ala Thr Tyr Leu Ser Gly Ala Gln Val Ser Thr Ile Thr  
 210 215 220  
 Pro Thr Ser Thr Arg Gln Thr Thr Ala Met Asp Phe Ser Tyr Ala Asn  
 225 230 235 240  
 Glu Thr Val Cys Trp Val His Val Gly Asp Ser Ala Ala Gln Thr Gln  
 245 250 255  
 Leu Lys Cys Ala Arg Met Pro Gly Leu Lys Gly Phe Val Asp Glu His  
 260 265 270  
 Thr Ile Asn Ile Ser Leu Ser Leu His His Val Glu Gln Met Ala Ile  
 275 280 285  
 Asp Trp Leu Thr Gly Asn Phe Tyr Phe Val Asp Asp Ile Asp Asp Arg  
 290 295 300  
 Ile Phe Val Cys Asn Arg Asn Gly Asp Thr Cys Val Thr Leu Leu Asp  
 305 310 315 320  
 Leu Glu Leu Tyr Asn Pro Lys Gly Ile Ala Leu Asp Pro Ala Met Gly  
 325 330 335  
 Lys Val Phe Phe Thr Asp Tyr Gly Gln Ile Pro Lys Val Glu Arg Cys  
 340 345 350  
 Asp Met Asp Gly Gln Asn Arg Thr Lys Leu Val Asp Ser Lys Ile Val  
 355 360 365  
 Phe Pro His Gly Ile Thr Leu Asp Leu Val Ser Arg Leu Val Tyr Trp  
 370 375 380  
 Ala Asp Ala Tyr Leu Asp Tyr Ile Glu Val Val Asp Tyr Glu Gly Lys  
 385 390 395 400  
 Gly Arg Gln Thr Ile Ile Gln Gly Ile Leu Ile Glu His Leu Tyr Gly  
 405 410 415  
 Leu Thr Val Phe Glu Asn Tyr Leu Tyr Ala Thr Asn Ser Asp Asn Ala  
 420 425 430  
 Asn Thr Gln Gln Lys Thr Ser Val Ile Arg Val Asn Arg Phe Asn Ser  
 435 440 445  
 Thr Glu Tyr Gln Val Val Thr Arg Val Asp Lys Gly Gly Ala Leu His  
 450 455 460

FIG. 12B

Ile Tyr His Gln Arg Arg Gln Pro Arg Val Arg Ser His Ala Cys Glu  
 465 470 475 480  
 Asn Asp Gln Tyr Gly Lys Pro Gly Gly Cys Ser Asp Ile Cys Leu Leu  
 485 490 495  
 Ala Asn Ser His Lys Ala Arg Thr Cys Arg Cys Arg Ser Gly Phe Ser  
 500 505 510  
 Leu Gly Ser Asp Gly Lys Ser Cys Lys Lys Pro Glu His Glu Leu Phe  
 515 520 525  
 Leu Val Tyr Gly Lys Gly Arg Pro Gly Ile Ile Arg Gly Met Asp Met  
 530 535 540  
 Gly Ala Lys Val Pro Asp Glu His Met Ile Pro Ile Glu Asn Leu Met  
 545 550 555 560  
 Asn Pro Arg Ala Leu Asp Phe His Ala Glu Thr Gly Phe Ile Tyr Phe  
 565 570 575  
 Ala Asp Thr Thr Ser Tyr Leu Ile Gly Arg Gln Lys Ile Asp Gly Thr  
 580 585 590  
 Glu Arg Glu Thr Ile Leu Lys Asp Gly Ile His Asn Val Glu Gly Val  
 595 600 605  
 Ala Val Asp Trp Met Gly Asp Asn Leu Tyr Trp Thr Asp Asp Gly Pro  
 610 615 620  
 Lys Lys Thr Ile Ser Val Ala Arg Leu Glu Lys Ala Ala Gln Thr Arg  
 625 630 635 640  
 Lys Thr Leu Ile Glu Gly Lys Met Thr His Pro Arg Ala Ile Val Val  
 645 650 655  
 Asp Pro Leu Asn Gly Trp Met Tyr Trp Thr Asp Trp Glu Glu Asp Pro  
 660 665 670  
 Lys Asp Ser Arg Arg Gly Arg Leu Glu Arg Ala Trp Met Asp Gly Ser  
 675 680 685  
 His Arg Asp Ile Phe Val Thr Ser Lys Thr Val Leu Trp Pro Asn Gly  
 690 695 700  
 Leu Ser Leu Asp Ile Pro Ala Gly Arg Leu Tyr Trp Val Asp Ala Phe  
 705 710 715 720  
 Tyr Asp Arg Ile Glu Thr Ile Leu Leu Asn Gly Thr Asp Arg Lys Ile  
 725 730 735  
 Val Tyr Glu Gly Pro Glu Leu Asn His Ala Phe Gly Leu Cys His His  
 740 745 750  
 Gly Asn Tyr Leu Phe Trp Thr Glu Tyr Arg Ser Gly Ser Val Tyr Arg  
 755 760 765  
 Leu Glu Arg Gly Val Ala Gly Ala Pro Pro Thr Val Thr Leu Leu Arg  
 770 775 780  
 Ser Glu Arg Pro Pro Ile Phe Glu Ile Arg Met Tyr Asp Ala His Glu  
 785 790 795 800  
 Gln Gln Val Gly Thr Asn Lys Cys Arg Val Asn Asn Gly Gly Cys Ser  
 805 810 815  
 Ser Leu Cys Leu Ala Thr Pro Gly Ser Arg Gln Cys Ala Cys Ala Glu  
 820 825 830  
 Asp Gln Val Leu Asp Thr Asp Gly Val Thr Cys Leu Ala Asn Pro Ser  
 835 840 845  
 Tyr Val Pro Pro Pro Gln Cys Gln Pro Gly Gln Phe Ala Cys Ala Asn  
 850 855 860  
 Asn Arg Cys Ile Gln Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys  
 865 870 875 880  
 Leu Asp Asn Ser Asp Glu Ala Pro Ala Leu Cys His Gln His Thr Cys  
 885 890 895  
 Pro Ser Asp Arg Phe Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg  
 900 905 910  
 Trp Leu Cys Asp Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser  
 915 920 925

FIG. 12B

Asn Ala Thr Cys Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys  
 930 935 940  
 Ala Ser Gly Arg Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp  
 945 950 955 960  
 Asp Cys Gly Asp Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro Thr  
 965 970 975  
 Cys Phe Pro Leu Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile Asn  
 980 985 990  
 Ile Asn Trp Arg Cys Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser Asp  
 995 1000 1005  
 Glu Ala Gly Cys Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys Asn  
 1010 1015 1020  
 Ser Gly Arg Cys Ile Pro Glu His Trp Thr Cys Asp Gly Asp Asn Asp  
 025 1030 1035 1040  
 Cys Gly Asp Tyr Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln Ala  
 1045 1050 1055  
 Thr Arg Pro Pro Gly Gly Cys His Ser Asp Glu Phe Gln Cys Pro Leu  
 1060 1065 1070  
 Asp Gly Leu Cys Ile Pro Leu Arg Trp Arg Cys Asp Gly Asp Thr Asp  
 1075 1080 1085  
 Cys Met Asp Ser Ser Asp Glu Lys Ser Cys Glu Gly Val Thr His Val  
 1090 1095 1100  
 Cys Asp Pro Asn Val Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys Ile  
 105 1110 1115 1120  
 Ser Lys Ala Trp Val Cys Asp Gly Asp Ser Asp Cys Glu Asp Asn Ser  
 1125 1130 1135  
 Asp Glu Glu Asn Cys Glu Ala Leu Ala Cys Arg Pro Pro Ser His Pro  
 1140 1145 1150  
 Cys Ala Asn Asn Thr Ser Val Cys Leu Pro Pro Asp Lys Leu Cys Asp  
 1155 1160 1165  
 Gly Lys Asp Asp Cys Gly Asp Gly Ser Asp Glu Gly Glu Leu Cys Asp  
 1170 1175 1180  
 Gln Cys Ser Leu Asn Asn Gly Gly Cys Ser His Asn Cys Ser Val Ala  
 185 1190 1195 1200  
 Pro Gly Glu Gly Ile Val Cys Ser Cys Pro Leu Gly Met Glu Leu Gly  
 1205 1210 1215  
 Ser Asp Asn His Thr Cys Gln Ile Gln Ser Tyr Cys Ala Lys His Leu  
 1220 1225 1230  
 Lys Cys Ser Gln Lys Cys Asp Gln Asn Lys Phe Ser Val Lys Cys Ser  
 1235 1240 1245  
 Cys Tyr Glu Gly Trp Val Leu Glu Pro Asp Gly Glu Thr Cys Arg Ser  
 1250 1255 1260  
 Leu Asp Pro Phe Lys Leu Phe Ile Ile Phe Ser Asn Arg His Glu Ile  
 265 1270 1275 1280  
 Arg Arg Ile Asp Leu His Lys Gly Asp Tyr Ser Val Leu Val Pro Gly  
 1285 1290 1295  
 Leu Arg Asn Thr Ile Ala Leu Asp Phe His Leu Ser Gln Ser Ala Leu  
 1300 1305 1310  
 Tyr Trp Thr Asp Ala Val Glu Asp Lys Ile Tyr Arg Gly Lys Leu Leu  
 1315 1320 1325  
 Asp Asn Gly Ala Leu Thr Ser Phe Glu Val Val Ile Gln Tyr Gly Leu  
 1330 1335 1340  
 Ala Thr Pro Glu Gly Leu Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr  
 345 1350 1355 1360  
 Trp Val Glu Ser Asn Leu Asp Gln Ile Glu Val Ala Lys Leu Asp Gly  
 1365 1370 1375  
 Thr Leu Arg Thr Thr Leu Leu Ala Gly Asp Ile Glu His Pro Arg Ala  
 1380 1385 1390  
 Ile Ala Leu Asp Pro Arg Asp Gly Ile Leu Phe Trp Thr Asp Trp Asp

FIG. 12B

	1395		1400		1405
Ala Ser Leu Pro Arg Ile Glu Ala Ala Ser Met Ser Gly Ala Gly Arg					
1410		1415		1420	
Arg Thr Ile His Arg Glu Thr Gly Ser Gly Gly Cys Ala Asn Gly Leu					
425	1430		1435		1440
Thr Val Asp Tyr Leu Glu Lys Arg Ile Leu Trp Ile Asp Ala Arg Ser					
	1445		1450		1455
Asp Ala Ile Tyr Ser Ala Arg Tyr Asp Gly Ser Gly His Met Glu Val					
1460		1465		1470	
Leu Arg Gly His Glu Phe Leu Ser His Pro Phe Ala Val Thr Leu Tyr					
1475		1480		1485	
Gly Gly Glu Val Tyr Trp Thr Asp Trp Arg Thr Asn Thr Leu Ala Lys					
1490	1495		1500		
Ala Asn Lys Trp Thr Gly His Asn Val Thr Val Val Gln Arg Thr Asn					
505	1510		1515		1520
Thr Gln Pro Phe Asp Leu Gln Val Tyr His Pro Ser Arg Gln Pro Met					
	1525		1530		1535
Ala Pro Asn Pro Cys Glu Ala Asn Gly Gly Arg Gly Pro Cys Ser His					
1540		1545		1550	
Leu Cys Leu Ile Asn Tyr Asn Arg Thr Val Ser Trp Ala Cys Pro His					
1555		1560		1565	
Leu Met Lys Leu His Lys Asp Asn Thr Thr Cys Tyr Glu Phe Lys Lys					
1570	1575		1580		
Phe Leu Leu Tyr Ala Arg Gln Met Glu Ile Arg Gly Val Asp Leu Asp					
585	1590		1595		1600
Ala Pro Tyr Tyr Asn Tyr Ile Ile Ser Phe Thr Val Pro Asp Ile Asp					
	1605		1610		1615
Asn Val Thr Val Leu Asp Tyr Asp Ala Arg Glu Gln Arg Val Tyr Trp					
1620		1625		1630	
Ser Asp Val Arg Thr Gln Ala Ile Lys Arg Ala Phe Ile Asn Gly Thr					
1635	1640		1645		
Gly Val Glu Thr Val Val Ser Ala Asp Leu Pro Asn Ala His Gly Leu					
1650	1655		1660		
Ala Val Asp Trp Val Ser Arg Asn Leu Phe Trp Thr Ser Tyr Asp Thr					
665	1670		1675		1680
Asn Lys Lys Gln Ile Asn Val Ala Arg Leu Asp Gly Ser Phe Lys Asn					
	1685		1690		1695
Ala Val Val Gln Gly Leu Glu Gln Pro His Gly Leu Val Val His Pro					
1700		1705		1710	
Leu Arg Gly Lys Leu Tyr Trp Thr Asp Gly Asp Asn Ile Ser Met Ala					
1715		1720		1725	
Asn Met Asp Gly Ser Asn His Thr Leu Leu Phe Ser Gly Gln Lys Gly					
1730	1735		1740		
Pro Val Gly Leu Ala Ile Asp Phe Pro Glu Ser Lys Leu Tyr Trp Ile					
745	1750		1755		1760
Ser Ser Gly Asn His Thr Ile Asn Arg Cys Asn Leu Asp Gly Ser Glu					
	1765		1770		1775
Leu Glu Val Ile Asp Thr Met Arg Ser Gln Leu Gly Lys Ala Thr Ala					
1780		1785		1790	
Leu Ala Ile Met Gly Asp Lys Leu Trp Trp Ala Asp Gln Val Ser Glu					
1795		1800		1805	
Lys Met Gly Thr Cys Asn Lys Ala Asp Gly Ser Gly Ser Val Val Leu					
1810	1815		1820		
Arg Asn Ser Thr Thr Leu Val Met His Met Lys Val Tyr Asp Glu Ser					
825	1830		1835		1840
Ile Gln Leu Glu His Glu Gly Thr Asn Pro Cys Ser Val Asn Asn Gly					
	1845		1850		1855
Asp Cys Ser Gln Leu Cys Leu Pro Thr Ser Glu Thr Thr Arg Ser Cys					
1860		1865		1870	

FIG. 12B

Met Cys Thr Ala Gly Tyr Ser Leu Arg Ser Gly Gln Gln Ala Cys Glu  
 1875 1880 1885  
 Gly Val Gly Ser Phe Leu Leu Tyr Ser Val His Glu Gly Ile Arg Gly  
 1890 1895 1900  
 Ile Pro Leu Asp Pro Asn Asp Lys Ser Asp Ala Leu Val Pro Val Ser  
 905 1910 1915 1920  
 Gly Thr Ser Leu Ala Val Gly Ile Asp Phe His Ala Glu Asn Asp Thr  
 1925 1930 1935  
 Ile Tyr Trp Val Asp Met Gly Leu Ser Thr Ile Ser Arg Ala Lys Arg  
 1940 1945 1950  
 Asp Gln Thr Trp Arg Glu Asp Val Val Thr Asn Gly Ile Gly Arg Val  
 1955 1960 1965  
 Glu Gly Ile Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp Thr Asp  
 1970 1975 1980  
 Gln Gly Phe Asp Val Ile Glu Val Ala Arg Leu Asn Gly Ser Phe Arg  
 985 1990 1995 2000  
 Tyr Val Val Ile Ser Gln Gly Leu Asp Lys Pro Arg Ala Ile Thr Val  
 2005 2010 2015  
 His Pro Glu Lys Gly Tyr Leu Phe Trp Thr Glu Trp Gly His Tyr Pro  
 2020 2025 2030  
 Arg Ile Glu Arg Ser Arg Leu Asp Gly Thr Glu Arg Val Val Leu Val  
 2035 2040 2045  
 Asn Val Ser Ile Ser Trp Pro Asn Gly Ile Ser Val Asp Tyr Gln Gly  
 2050 2055 2060  
 Gly Lys Leu Tyr Trp Cys Asp Ala Arg Met Asp Lys Ile Glu Arg Ile  
 065 2070 2075 2080  
 Asp Leu Glu Thr Gly Glu Asn Arg Glu Val Val Leu Ser Ser Asn Asn  
 2085 2090 2095  
 Met Asp Met Phe Ser Val Ser Val Phe Glu Asp Phe Ile Tyr Trp Ser  
 2100 2105 2110  
 Asp Arg Thr His Ala Asn Gly Ser Ile Lys Arg Gly Cys Lys Asp Asn  
 2115 2120 2125  
 Ala Thr Asp Ser Val Pro Leu Arg Thr Gly Ile Gly Val Gln Leu Lys  
 2130 2135 2140  
 Asp Ile Lys Val Phe Asn Arg Asp Arg Gln Lys Gly Thr Asn Val Cys  
 145 2150 2155 2160  
 Ala Val Ala Asn Gly Gly Cys Gln Gln Leu Cys Leu Tyr Arg Gly Gly  
 2165 2170 2175  
 Gly Gln Arg Ala Cys Ala Cys Ala His Gly Met Leu Ala Glu Asp Gly  
 2180 2185 2190  
 Ala Ser Cys Arg Glu Tyr Ala Gly Tyr Leu Leu Tyr Ser Glu Arg Thr  
 2195 2200 2205  
 Ile Leu Lys Ser Ile His Leu Ser Asp Glu Arg Asn Leu Asn Ala Pro  
 2210 2215 2220  
 Val Gln Pro Phe Glu Asp Pro Glu His Met Lys Asn Val Ile Ala Leu  
 225 2230 2235 2240  
 Ala Phe Asp Tyr Arg Ala Gly Thr Ser Pro Gly Thr Pro Asn Arg Ile  
 2245 2250 2255  
 Phe Phe Ser Asp Ile His Phe Gly Asn Ile Gln Gln Ile Asn Asp Asp  
 2260 2265 2270  
 Gly Ser Gly Arg Thr Thr Ile Val Glu Asn Val Gly Ser Val Glu Gly  
 2275 2280 2285  
 Leu Ala Tyr His Arg Gly Trp Asp Thr Leu Tyr Trp Thr Ser Tyr Thr  
 2290 2295 2300  
 Thr Ser Thr Ile Thr Arg His Thr Val Asp Gln Thr Arg Pro Gly Ala  
 305 2310 2315 2320  
 Phe Glu Arg Glu Thr Val Ile Thr Met Ser Gly Asp Asp His Pro Arg  
 2325 2330 2335  
 Ala Phe Val Leu Asp Glu Cys Gln Asn Leu Met Phe Trp Thr Asn Trp

FIG. 12B

Asn	Glu	Leu	His	Pro	Ser	Ile	Met	Arg	Ala	Ala	Leu	Ser	Gly	Ala	Asn	2340	2345	2350
Val	Leu	Thr	Leu	Ile	Glu	Lys	Asp	Ile	Arg	Thr	Pro	Asn	Gly	Leu	Ala	2355	2360	2365
Ile	Asp	His	Arg	Ala	Glu	Lys	Leu	Tyr	Phe	Ser	Asp	Ala	Thr	Leu	Asp	2370	2375	2380
Lys	Ile	Glu	Arg	Cys	Glu	Tyr	Asp	Gly	Ser	His	Arg	Tyr	Val	Ile	Leu	385	2390	2400
Lys	Ser	Glu	Pro	Val	His	Pro	Phe	Gly	Leu	Ala	Val	Tyr	Gly	Glu	His	2405	2410	2415
Ile	Phe	Trp	Thr	Asp	Trp	Val	Arg	Arg	Ala	Val	Gln	Arg	Ala	Asn	Lys	2420	2425	2430
Tyr	Val	Gly	Ser	Asp	Met	Lys	Leu	Leu	Arg	Val	Asp	Ile	Pro	Gln	Gln	2435	2440	2445
Pro	Met	Gly	Ile	Ile	Ala	Val	Ala	Asn	Asp	Thr	Asn	Ser	Cys	Glu	Leu	2450	2455	2460
Ser	Pro	Cys	Arg	Ile	Asn	Asn	Gly	Gly	Cys	Gln	Asp	Leu	Cys	Leu	Leu	465	2470	2475
Thr	His	Gln	Gly	His	Val	Asn	Cys	Ser	Cys	Arg	Gly	Gly	Arg	Ile	Leu	2485	2490	2495
Gln	Glu	Asp	Phe	Thr	Cys	Arg	Ala	Val	Asn	Ser	Ser	Cys	Arg	Ala	Gln	2500	2505	2510
Asp	Glu	Phe	Glu	Cys	Ala	Asn	Gly	Glu	Cys	Ile	Ser	Phe	Ser	Leu	Thr	2515	2520	2525
Cys	Asp	Gly	Val	Ser	His	Cys	Lys	Asp	Lys	Ser	Asp	Glu	Lys	Pro	Ser	2530	2535	2540
Tyr	Cys	Asn	Ser	Arg	Arg	Cys	Lys	Lys	Thr	Phe	Arg	Gln	Cys	Asn	Asn	545	2550	2555
Gly	Arg	Cys	Val	Ser	Asn	Met	Leu	Trp	Cys	Asn	Gly	Val	Asp	Tyr	Cys	2565	2570	2575
Gly	Asp	Gly	Ser	Asp	Glu	Ile	Pro	Cys	Asn	Lys	Thr	Ala	Cys	Gly	Val	2580	2585	2590
Gly	Glu	Phe	Arg	Cys	Arg	Asp	Gly	Ser	Cys	Ile	Gly	Asn	Ser	Ser	Arg	2595	2600	2605
Cys	Asn	Gln	Phe	Val	Asp	Cys	Glu	Asp	Ala	Ser	Asp	Glu	Met	Asn	Cys	2610	2615	2620
Ser	Ala	Thr	Asp	Cys	Ser	Ser	Tyr	Phe	Arg	Leu	Gly	Val	Lys	Gly	Val	625	2630	2635
Leu	Phe	Gln	Pro	Cys	Glu	Arg	Thr	Ser	Leu	Cys	Tyr	Ala	Pro	Ser	Trp	2645	2650	2655
Val	Cys	Asp	Gly	Ala	Asn	Asp	Cys	Gly	Asp	Tyr	Ser	Asp	Glu	Arg	Asp	2660	2665	2670
Cys	Pro	Gly	Val	Lys	Arg	Pro	Arg	Cys	Pro	Leu	Asn	Tyr	Phe	Ala	Cys	2675	2680	2685
Pro	Ser	Gly	Arg	Cys	Ile	Pro	Met	Ser	Trp	Thr	Cys	Asp	Lys	Glu	Asp	2690	2695	2700
Asp	Cys	Glu	Asn	Gly	Glu	Asp	Glu	Thr	His	Cys	Asn	Lys	Phe	Cys	Ser	705	2710	2715
Glu	Ala	Gln	Phe	Glu	Cys	Gln	Asn	His	Arg	Cys	Ile	Ser	Lys	Gln	Trp	2725	2730	2735
Leu	Cys	Asp	Gly	Ser	Asp	Asp	Cys	Gly	Asp	Gly	Ser	Asp	Glu	Ala	Ala	2740	2745	2750
His	Cys	Glu	Gly	Lys	Thr	Cys	Gly	Pro	Ser	Ser	Phe	Ser	Cys	Pro	Gly	2755	2760	2765
Thr	His	Val	Cys	Val	Pro	Glu	Arg	Trp	Leu	Cys	Asp	Gly	Asp	Lys	Asp	2770	2775	2780
Cys	Thr	Asp	Gly	Ala	Asp	Glu	Ser	Val	Thr	Ala	Gly	Cys	Leu	Tyr	Asn	785	2790	2795
																2805	2810	2815

FIG. 12B

Ser Thr Cys Asp Asp Arg Glu Phe Met Cys Gln Asn Arg Leu Cys Ile  
 2820 2825 2830  
 Pro Lys His Phe Val Cys Asp His Asp Arg Asp Cys Ala Asp Gly Ser  
 2835 2840 2845  
 Asp Glu Ser Pro Glu Cys Glu Tyr Pro Thr Cys Gly Pro Asn Glu Phe  
 2850 2855 2860  
 Arg Cys Ala Asn Gly Arg Cys Leu Ser Ser Arg Gln Trp Glu Cys Asp  
 865 2870 2875 2880  
 Gly Glu Asn Asp Cys His Asp His Ser Asp Glu Ala Pro Lys Asn Pro  
 2885 2890 2895  
 His Cys Thr Ser Pro Glu His Lys Cys Asn Ala Ser Ser Gln Phe Leu  
 2900 2905 2910  
 Cys Ser Ser Gly Arg Cys Val Ala Glu Ala Leu Leu Cys Asn Gly Gln  
 2915 2920 2925  
 Asp Asp Cys Gly Asp Gly Ser Asp Glu Arg Gly Cys His Val Asn Glu  
 2930 2935 2940  
 Cys Leu Ser Arg Lys Leu Ser Gly Cys Ser Gln Asp Cys Glu Asp Leu  
 945 2950 2955 2960  
 Lys Ile Gly Phe Lys Cys Arg Cys Arg Pro Gly Phe Arg Leu Lys Asp  
 2965 2970 2975  
 Asp Gly Arg Thr Cys Ala Asp Leu Asp Glu Cys Ser Thr Thr Phe Pro  
 2980 2985 2990  
 Cys Ser Gln Leu Cys Ile Asn Thr His Gly Ser Tyr Lys Cys Leu Cys  
 2995 3000 3005  
 Val Glu Gly Tyr Ala Pro Arg Gly Gly Asp Pro His Ser Cys Lys Ala  
 3010 3015 3020  
 Val Thr Asp Glu Glu Pro Phe Leu Ile Phe Ala Asn Arg Tyr Tyr Leu  
 025 3030 3035 3040  
 Arg Lys Leu Asn Leu Asp Gly Ser Asn Tyr Thr Leu Leu Lys Gln Gly  
 3045 3050 3055  
 Leu Asn Asn Ala Val Ala Leu Ala Phe Asp Tyr Arg Glu Gln Met Ile  
 3060 3065 3070  
 Tyr Trp Thr Gly Val Thr Thr Gln Gly Ser Met Ile Arg Arg Met His  
 3075 3080 3085  
 Leu Asn Gly Ser Asn Val Gln Val Leu His Arg Thr Gly Leu Ser Asn  
 3090 3095 3100  
 Pro Asp Gly Leu Ala Val Asp Trp Val Gly Gly Asn Leu Tyr Trp Cys  
 105 3110 3115 3120  
 Asp Lys Gly Arg Asp Thr Ile Glu Val Ser Lys Leu Asn Gly Ala Tyr  
 3125 3130 3135  
 Arg Thr Val Leu Val Ser Ser Gly Leu Arg Glu Pro Arg Ala Leu Val  
 3140 3145 3150  
 Val Asp Val Gln Asn Gly Tyr Leu Tyr Trp Thr Asp Trp Gly Asp His  
 3155 3160 3165  
 Ser Leu Ile Gly Arg Ile Gly Met Asp Gly Ser Gly Arg Ser Ile Ile  
 3170 3175 3180  
 Val Asp Thr Lys Ile Thr Trp Pro Asn Gly Leu Thr Val Asp Tyr Val  
 185 3190 3195 3200  
 Thr Glu Arg Ile Tyr Trp Ala Asp Ala Arg Glu Asp Tyr Ile Glu Phe  
 3205 3210 3215  
 Ala Ser Leu Asp Gly Ser Asn Arg His Val Val Leu Ser Gln Asp Ile  
 3220 3225 3230  
 Pro His Ile Phe Ala Leu Thr Leu Phe Glu Asp Tyr Val Tyr Trp Thr  
 3235 3240 3245  
 Asp Trp Glu Thr Lys Ser Ile Asn Arg Ala His Lys Thr Thr Gly Ala  
 3250 3255 3260  
 Asn Lys Thr Leu Leu Ile Ser Thr Leu His Arg Pro Met Asp Leu His  
 265 3270 3275 3280  
 Val Phe His Ala Leu Arg Gln Pro Asp Val Pro Asn His Pro Cys Lys

FIG. 12B



Arg Cys Asn Met Phe Asp Asp Cys Gly Asp Gly Ser Asp Glu Glu Asp  
 3765 3770 3775  
 Cys Ser Ile Asp Pro Lys Leu Thr Ser Cys Ala Thr Asn Ala Ser Met  
 3780 3785 3790  
 Cys Gly Asp Glu Ala Arg Cys Val Arg Thr Glu Lys Ala Ala Tyr Cys  
 3795 3800 3805  
 Ala Cys Arg Ser Gly Phe His Thr Val Pro Gly Gln Pro Gly Cys Gln  
 3810 3815 3820  
 Asp Ile Asn Glu Cys Leu Arg Phe Gly Thr Cys Ser Gln Leu Trp Asn  
 825 3830 3835 3840  
 Lys Pro Lys Gly Gly His Leu Cys Ser Cys Ala Arg Asn Phe Met Lys  
 3845 3850 3855  
 Thr His Asn Thr Cys Lys Ala Glu Gly Ser Glu Tyr Gln Val Leu Tyr  
 3860 3865 3870  
 Ile Ala Asp Asp Asn Glu Ile Arg Ser Leu Phe Pro Gly His Pro His  
 3875 3880 3885  
 Ser Ala Tyr Glu Gln Thr Phe Gln Gly Asp Glu Ser Val Arg Ile Asp  
 3890 3895 3900  
 Ala Met Asp Val His Val Lys Ala Gly Arg Val Tyr Trp Thr Asn Trp  
 905 3910 3915 3920  
 His Thr Gly Thr Ile Ser Tyr Arg Ser Leu Pro Pro Ala Ala Pro Pro  
 3925 3930 3935  
 Thr Thr Ser Asn Arg His Arg Arg Gln Ile Asp Arg Gly Val Thr His  
 3940 3945 3950  
 Leu Asn Ile Ser Gly Leu Lys Met Pro Arg Gly Ile Ala Ile Asp Trp  
 3955 3960 3965  
 Val Ala Gly Asn Val Tyr Trp Thr Asp Ser Gly Arg Asp Val Ile Glu  
 3970 3975 3980  
 Val Ala Gln Met Lys Gly Glu Asn Arg Lys Thr Leu Ile Ser Gly Met  
 985 3990 3995 4000  
 Ile Asp Glu Pro His Ala Ile Val Val Asp Pro Leu Arg Gly Thr Met  
 4005 4010 4015  
 Tyr Trp Ser Asp Trp Gly Asn His Pro Lys Ile Glu Thr Ala Ala Met  
 4020 4025 4030  
 Asp Gly Thr Leu Arg Glu Thr Leu Val Gln Asp Asn Ile Gln Trp Pro  
 4035 4040 4045  
 Thr Gly Leu Ala Val Asp Tyr His Asn Glu Arg Leu Tyr Trp Ala Asp  
 4050 4055 4060  
 Ala Lys Leu Ser Val Ile Gly Ser Ile Arg Leu Asn Gly Thr Asp Pro  
 065 4070 4075 4080  
 Ile Val Ala Ala Asp Ser Lys Arg Gly Leu Ser His Pro Phe Ser Ile  
 4085 4090 4095  
 Asp Val Phe Glu Asp Tyr Ile Tyr Gly Val Thr Tyr Ile Asn Asn Arg  
 4100 4105 4110  
 Val Phe Lys Ile His Lys Phe Gly His Ser Pro Leu Tyr Asn Leu Thr  
 4115 4120 4125  
 Gly Gly Leu Ser His Ala Ser Asp Val Val Leu Tyr His Gln His Lys  
 4130 4135 4140  
 Gln Pro Glu Val Thr Asn Pro Cys Asp Arg Lys Lys Cys Glu Trp Leu  
 145 4150 4155 4160  
 Cys Leu Leu Ser Pro Ser Gly Pro Val Cys Thr Cys Pro Asn Gly Lys  
 4165 4170 4175  
 Arg Leu Asp Asn Gly Thr Cys Val Pro Val Pro Ser Pro Thr Pro Pro  
 4180 4185 4190  
 Pro Asp Ala Pro Arg Pro Gly Thr Cys Thr Leu Gln Cys Phe Asn Gly  
 4195 4200 4205  
 Gly Ser Cys Phe Leu Asn Ala Arg Arg Gln Pro Lys Cys Arg Cys Gln  
 4210 4215 4220  
 Pro Arg Tyr Thr Gly Asp Lys Cys Glu Leu Asp Gln Cys Trp Glu Tyr

FIG. 12B

225  
 Cys His Asn Gly Gly Thr Cys Ala Ala Ser Pro Ser Gly Met Pro Thr  
 4230  
 4245  
 Cys Arg Cys Pro Thr Gly Phe Thr Gly Pro Lys Cys Thr Ala Gln Val  
 4250  
 4260  
 Cys Ala Gly Tyr Cys Ser Asn Asn Ser Thr Cys Thr Val Asn Gln Gly  
 4275  
 4280  
 4285  
 Asn Gln Pro Gln Cys Arg Cys Leu Pro Gly Phe Leu Gly Asp Arg Cys  
 4290  
 4295  
 4300  
 Gln Tyr Arg Gln Cys Ser Gly Phe Cys Glu Asn Phe Gly Thr Cys Gln  
 305  
 4310  
 4315  
 4320  
 Met Ala Ala Asp Gly Ser Arg Gln Cys Arg Cys Thr Val Tyr Phe Glu  
 4325  
 4330  
 4335  
 Gly Pro Arg Cys Glu Val Asn Lys Cys Ser Arg Cys Leu Gln Gly Ala  
 4340  
 4345  
 4350  
 Cys Val Val Asn Lys Gln Thr Gly Asp Val Thr Cys Asn Cys Thr Asp  
 4355  
 4360  
 4365  
 Gly Arg Val Ala Pro Ser Cys Leu Thr Cys Ile Asp His Cys Ser Asn  
 4370  
 4375  
 4380  
 Gly Gly Ser Cys Thr Met Asn Ser Lys Met Met Pro Glu Cys Gln Cys  
 385  
 4390  
 4395  
 4400  
 Pro Pro His Met Thr Gly Pro Arg Cys Gln Glu Gln Val Val Ser Gln  
 4405  
 4410  
 4415  
 Gln Gln Pro Gly His Met Ala Ser Ile Leu Ile Pro Leu Leu Leu Leu  
 4420  
 4425  
 4430  
 Leu Leu Leu Leu Leu Val Ala Gly Val Val Phe Trp Tyr Lys Arg Arg  
 4435  
 4440  
 4445  
 Val Arg Gly Ala Lys Gly Phe Gln His Gln Arg Met Thr Asn Gly Ala  
 4450  
 4455  
 4460  
 Met Asn Val Glu Ile Gly Asn Pro Thr Tyr Lys Met Tyr Glu Gly Gly  
 465  
 4470  
 4475  
 4480  
 Glu Pro Asp Asp Val Gly Gly Leu Leu Asp Ala Asp Phe Ala Leu Asp  
 4485  
 4490  
 4495  
 Pro Asp Lys Pro Thr Asn Phe Thr Asn Pro Val Tyr Ala Thr Leu Tyr  
 4500  
 4505  
 4510  
 Met Gly Gly His Gly Ser Arg His Ser Leu Ala Ser Thr Asp Glu Lys  
 4515  
 4520  
 4525  
 Arg Glu Leu Leu Gly Arg Gly Pro Glu Asp Glu Ile Gly Asp Pro Leu  
 4530  
 4535  
 4540  
 Ala  
 545

FIG. 12B

GCTACAATCC ATCTGGTCTC CTCCAGCTCC TTCTTTCTGC AAC ATG GGG AAG AAC	55
Met Gly Lys Asn 1	
AAA CTC CTT CAT CCA AGT CTG GTT CTT CTC CTC TTG GTC CTC CTG CCC	103
Lys Leu Leu His Pro Ser Leu Val Leu Leu Leu Leu Val Leu Leu Pro 5 10 15 20	
ACA GAC GCC TCA GTC TCT GGA AAA CCG CAG TAT ATG GTT CTG GTC CCC	151
Thr Asp Ala Ser Val Ser Gly Lys Pro Gln Tyr Met Val Leu Val Pro 25 30 35	
TCC CTG CTC CAC ACT GAG ACC ACT GAG AAG GGC TGT GTC CTT CTG AGC	199
Ser Leu Leu His Thr Glu Thr Thr Glu Lys Gly Cys Val Leu Leu Ser 40 45 50	
TAC CTG AAT GAG ACA GTG ACT GTA AGT GCT TCC TTG GAG TCT GTC AGG	247
Tyr Leu Asn Glu Thr Val Thr Val Ser Ala Ser Leu Glu Ser Val Arg 55 60 65	
GGA AAC AGG AGC CTC TTC ACT GAC CTG GAG GCG GAG AAT GAC GTA CTC	295
Gly Asn Arg Ser Leu Phe Thr Asp Leu Glu Ala Glu Asn Asp Val Leu 70 75 80	
CAC TGT GTC GCC TTC GCT GTC CCA AAG TCT TCA TCC AAT GAG GAG GTA	343
His Cys Val Ala Phe Ala Val Pro Lys Ser Ser Ser Asn Glu Glu Val 85 90 95 100	
ATG TTC CTC ACT GTC CAA GTG AAA GGA CCA ACC CAA GAA TTT AAG AAG	391
Met Phe Leu Thr Val Gln Val Lys Gly Pro Thr Gln Glu Phe Lys Lys 105 110 115	
CGG ACC ACA GTG ATG GTT AAG AAC GAG GAC AGT CTG GTC TTT GTC CAG	439
Arg Thr Thr Val Met Val Lys Asn Glu Asp Ser Leu Val Phe Val Gln 120 125 130	
ACA GAC AAA TCA ATC TAC AAA CCA GGG CAG ACA GTG AAA TTT CGT GTT	487
Thr Asp Lys Ser Ile Tyr Lys Pro Gly Gln Thr Val Lys Phe Arg Val 135 140 145	
GTC TCC ATG GAT GAA AAC TTT CAC CCC CTG AAT GAG TTG ATT CCA CTA	535
Val Ser Met Asp Glu Asn Phe His Pro Leu Asn Glu Leu Ile Pro Leu 150 155 160	
GTA TAC ATT CAG GAT CCC AAA GGA AAT CGC ATC GCA CAA TGG CAG AGT	583
Val Tyr Ile Gln Asp Pro Lys Gly Asn Arg Ile Ala Gln Trp Gln Ser 165 170 175 180	
TTC CAG TTA GAG GGT GGC CTC AAG CAA TTT TCT TTT CCC CTC TCA TCA	631
Phe Gln Leu Glu Gly Gly Leu Lys Gln Phe Ser Phe Pro Leu Ser Ser 185 190 195	
GAG CCC TTC CAG GGC TCC TAC AAG GTG GTG GTA CAG AAG AAA TCA GGT	679
Glu Pro Phe Gln Gly Ser Tyr Lys Val Val Val Gln Lys Lys Ser Gly 200 205 210	
GGA AGG ACA GAG CAC CCT TTC ACC GTG GAG GAA TTT GTT CTT CCC AAG	727

FIG. 13A

Gly	Arg	Thr	Glu	His	Pro	Phe	Thr	Val	Glu	Glu	Phe	Val	Leu	Pro	Lys		
		215					220					225					
TTT	GAA	GTA	CAA	GTA	ACA	GTG	CCA	AAG	ATA	ATC	ACC	ATC	TTG	GAA	GAA	775	
Phe	Glu	Val	Gln	Val	Thr	Val	Pro	Lys	Ile	Ile	Thr	Ile	Leu	Glu	Glu		
	230					235					240						
GAG	ATG	AAT	GTA	TCA	GTG	TGT	GGC	CTA	TAC	ACA	TAT	GGG	AAG	CCT	GTC	823	
Glu	Met	Asn	Val	Ser	Val	Cys	Gly	Leu	Tyr	Thr	Tyr	Gly	Lys	Pro	Val		
	245				250					255					260		
CCT	GGA	CAT	GTG	ACT	GTG	AGC	ATT	TGC	AGA	AAG	TAT	AGT	GAC	GCT	TCC	871	
Pro	Gly	His	Val	Thr	Val	Ser	Ile	Cys	Arg	Lys	Tyr	Ser	Asp	Ala	Ser		
				265					270					275			
GAC	TGC	CAC	GGT	GAA	GAT	TCA	CAG	GCT	TTC	TGT	GAG	AAA	TTC	AGT	GGA	919	
Asp	Cys	His	Gly	Glu	Asp	Ser	Gln	Ala	Phe	Cys	Glu	Lys	Phe	Ser	Gly		
			280					285					290				
CAG	CTA	AAC	AGC	CAT	GGC	TGC	TTC	TAT	CAG	CAA	GTA	AAA	ACC	AAG	GTC	967	
Gln	Leu	Asn	Ser	His	Gly	Cys	Phe	Tyr	Gln	Gln	Val	Lys	Thr	Lys	Val		
		295				300						305					
TTC	CAG	CTG	AAG	AGG	AAG	GAG	TAT	GAA	ATG	AAA	CTT	CAC	ACT	GAG	GCC	1015	
Phe	Gln	Leu	Lys	Arg	Lys	Glu	Tyr	Glu	Met	Lys	Leu	His	Thr	Glu	Ala		
	310					315					320						
CAG	ATC	CAA	GAA	GAA	GGA	ACA	GTG	GTG	GAA	TTG	ACT	GGA	AGG	CAG	TCC	1063	
Gln	Ile	Gln	Glu	Glu	Gly	Thr	Val	Val	Glu	Leu	Thr	Gly	Arg	Gln	Ser		
	325				330					335					340		
AGT	GAA	ATC	ACA	AGA	ACC	ATA	ACC	AAA	CTC	TCA	TTT	GTG	AAA	GTG	GAC	1111	
Ser	Glu	Ile	Thr	Arg	Thr	Ile	Thr	Lys	Leu	Ser	Phe	Val	Lys	Val	Asp		
				345					350					355			
TCA	CAC	TTT	CGA	CAG	GGA	ATT	CCC	TTC	TTT	GGG	CAG	GTG	CGC	CTA	GTA	1159	
Ser	His	Phe	Arg	Gln	Gly	Ile	Pro	Phe	Phe	Gly	Gln	Val	Arg	Leu	Val		
			360				365						370				
GAT	GGG	AAA	GGC	GTC	CCT	ATA	CCA	AAT	AAA	GTC	ATA	TTC	ATC	AGA	GGA	1207	
Asp	Gly	Lys	Gly	Val	Pro	Ile	Pro	Asn	Lys	Val	Ile	Phe	Ile	Arg	Gly		
		375					380					385					
AAT	GAA	GCA	AAC	TAT	TAC	TCC	AAT	GCT	ACC	ACG	GAT	GAG	CAT	GGC	CTT	1255	
Asn	Glu	Ala	Asn	Tyr	Tyr	Ser	Asn	Ala	Thr	Thr	Asp	Glu	His	Gly	Leu		
	390					395					400						
GTA	CAG	TTC	TCT	ATC	AAC	ACC	ACC	AAC	GTT	ATG	GGT	ACC	TCT	CTT	ACT	1303	
Val	Gln	Phe	Ser	Ile	Asn	Thr	Thr	Asn	Val	Met	Gly	Thr	Ser	Leu	Thr		
	405				410					415					420		
GTT	AGG	GTC	AAT	TAC	AAG	GAT	CGT	AGT	CCC	TGT	TAC	GGC	TAC	CAG	TGG	1351	
Val	Arg	Val	Asn	Tyr	Lys	Asp	Arg	Ser	Pro	Cys	Tyr	Gly	Tyr	Gln	Trp		
				425					430					435			
GTG	TCA	GAA	GAA	CAC	GAA	GAG	GCA	CAT	CAC	ACT	GCT	TAT	CTT	GTG	TTC	1399	
Val	Ser	Glu	Glu	His	Glu	Glu	Ala	His	His	Thr	Ala	Tyr	Leu	Val	Phe		
			440					445					450				

FIG. 13A

TCC CCA AGC AAG AGC TTT GTC CAC CTT GAG CCC ATG TCT CAT GAA CTA Ser Pro Ser Lys Ser Phe Val His Leu Glu Pro Met Ser His Glu Leu 455 460 465	1447
CCC TGT GGC CAT ACT CAG ACA GTC CAG GCA CAT TAT ATT CTG AAT GGA Pro Cys Gly His Thr Gln Thr Val Gln Ala His Tyr Ile Leu Asn Gly 470 475 480	1495
GGC ACC CTG CTG GGG CTG AAG AAG CTC TCC TTT TAT TAT CTG ATA ATG Gly Thr Leu Leu Gly Leu Lys Lys Leu Ser Phe Tyr Tyr Leu Ile Met 485 490 495 500	1543
GCA AAG GGA GGC ATT GTC CGA ACT GGG ACT CAT GGA CTG CTT GTG AAG Ala Lys Gly Gly Ile Val Arg Thr Gly Thr His Gly Leu Leu Val Lys 505 510 515	1591
CAG GAA GAC ATG AAG GGC CAT TTT TCC ATC TCA ATC CCT GTG AAG TCA Gln Glu Asp Met Lys Gly His Phe Ser Ile Ser Ile Pro Val Lys Ser 520 525 530	1639
GAC ATT GCT CCT GTC GCT CGG TTG CTC ATC TAT GCT GTT TTA CCT ACC Asp Ile Ala Pro Val Ala Arg Leu Leu Ile Tyr Ala Val Leu Pro Thr 535 540 545	1687
GGG GAC GTG ATT GGG GAT TCT GCA AAA TAT GAT GTT GAA AAT TGT CTG Gly Asp Val Ile Gly Asp Ser Ala Lys Tyr Asp Val Glu Asn Cys Leu 550 555 560	1735
GCC AAC AAG GTG GAT TTG AGC TTC AGC CCA TCA CAA AGT CTC CCA GCC Ala Asn Lys Val Asp Leu Ser Phe Ser Pro Ser Gln Ser Leu Pro Ala 565 570 575 580	1783
TCA CAC GCC CAC CTG CGA GTC ACA GCG GCT CCT CAG TCC GTC TGC GCC Ser His Ala His Leu Arg Val Thr Ala Ala Pro Gln Ser Val Cys Ala 585 590 595	1831
CTC CGT GCT GTG GAC CAA AGC GTG CTG CTC ATG AAG CCT GAT GCT GAG Leu Arg Ala Val Asp Gln Ser Val Leu Leu Met Lys Pro Asp Ala Glu 600 605 610	1879
CTC TCG GCG TCC TCG GTT TAC AAC CTG CTA CCA GAA AAG GAC CTC ACT Leu Ser Ala Ser Ser Val Tyr Asn Leu Leu Pro Glu Lys Asp Leu Thr 615 620 625	1927
GGC TTC CCT GGG CCT TTG AAT GAC CAG GAC GAT GAA GAC TGC ATC AAT Gly Phe Pro Gly Pro Leu Asn Asp Gln Asp Asp Glu Asp Cys Ile Asn 630 635 640	1975
CGT CAT AAT GTC TAT ATT AAT GGA ATC ACA TAT ACT CCA GTA TCA AGT Arg His Asn Val Tyr Ile Asn Gly Ile Thr Tyr Thr Pro Val Ser Ser 645 650 655 660	2023
ACA AAT GAA AAG GAT ATG TAC AGC TTC CTA GAG GAC ATG GGC TTA AAG Thr Asn Glu Lys Asp Met Tyr Ser Phe Leu Glu Asp Met Gly Leu Lys 665 670 675	2071
GCA TTC ACC AAC TCA AAG ATT CGT AAA CCC AAA ATG TGT CCA CAG CTT Ala Phe Thr Asn Ser Lys Ile Arg Lys Pro Lys Met Cys Pro Gln Leu 680 685 690	2119

FIG. 13A

CAA Gln	CAG Gln	TAT Tyr	GAA Glu	ATG Met	CAT His	GGA Gly	CCT Pro	GAA Glu	GGT Gly	CTA Leu	CGT Arg	GTA Val	GGT Gly	TTT Phe	TAT Tyr	2167
		695					700					705				
GAG Glu	TCA Ser	GAT Asp	GTA Val	ATG Met	GGA Gly	AGA Arg	GGC Gly	CAT His	GCA Ala	CGC Arg	CTG Leu	GTG Val	CAT His	GTT Val	GAA Glu	2215
	710					715					720					
GAG Glu	CCT Pro	CAC His	ACG Thr	GAG Glu	ACC Thr	GTA Val	CGA Arg	AAG Lys	TAC Tyr	TTC Phe	CCT Pro	GAG Glu	ACA Thr	TGG Trp	ATC Ile	2263
725					730					735					740	
TGG Trp	GAT Asp	TTG Leu	GTG Val	GTG Val	GTA Val	AAC Asn	TCA Ser	GCA Ala	GGG Gly	GTG Val	GCT Ala	GAG Glu	GTA Val	GGA Gly	GTA Val	2311
					745				750					755		
ACA Thr	GTC Val	CCT Pro	GAC Asp	ACC Thr	ATC Ile	ACC Thr	GAG Glu	TGG Trp	AAG Lys	GCA Ala	GGG Gly	GCC Ala	TTC Phe	TGC Cys	CTG Leu	2359
			760						765				770			
TCT Ser	GAA Glu	GAT Asp	GCT Ala	GGA Gly	CTT Leu	GGT Gly	ATC Ile	TCT Ser	TCC Ser	ACT Thr	GCC Ala	TCT Ser	CTC Leu	CGA Arg	GCC Ala	2407
		775					780						785			
TTC Phe	CAG Gln	CCC Pro	TTC Phe	TTT Phe	GTG Val	GAG Glu	CTT Leu	ACA Thr	ATG Met	CCT Pro	TAC Tyr	TCT Ser	GTG Val	ATT Ile	CGT Arg	2455
	790					795					800					
GGA Gly	GAG Glu	GCC Ala	TTC Phe	ACA Thr	CTC Leu	AAG Lys	GCC Ala	ACG Thr	GTC Val	CTA Leu	AAC Asn	TAC Tyr	CTT Leu	CCC Pro	AAA Lys	2503
805					810					815					820	
TGC Cys	ATC Ile	CGG Arg	GTC Val	AGT Ser	GTG Val	CAG Gln	CTG Leu	GAA Glu	GCC Ala	TCT Ser	CCC Pro	GCC Ala	TTC Phe	CTT Leu	GCT Ala	2551
				825					830					835		
GTC Val	CCA Pro	GTG Val	GAG Glu	AAG Lys	GAA Glu	CAA Gln	GCG Ala	CCT Pro	CAC His	TGC Cys	ATC Ile	TGT Cys	GCA Ala	AAC Asn	GGG Gly	2599
			840					845					850			
CGG Arg	CAA Gln	ACT Thr	GTG Val	TCC Ser	TGG Trp	GCA Ala	GTA Val	ACC Thr	CCA Pro	AAG Lys	TCA Ser	TTA Leu	GGA Gly	AAT Asn	GTG Val	2647
		855					860					865				
AAT Asn	TTC Phe	ACT Thr	GTG Val	AGC Ser	GCA Ala	GAG Glu	GCA Ala	CTA Leu	GAG Glu	TCT Ser	CAA Gln	GAG Glu	CTG Leu	TGT Cys	GGG Gly	2695
	870					875					880					
ACT Thr	GAG Glu	GTG Val	CCT Pro	TCA Ser	GTT Val	CCT Pro	GAA Glu	CAC His	GGA Gly	AGG Arg	AAA Lys	GAC Asp	ACA Thr	GTC Val	ATC Ile	2743
885					890					895					900	
AAG Lys	CCT Pro	CTG Leu	TTG Leu	GTT Val	GAA Glu	CCT Pro	GAA Glu	GGA Gly	CTA Leu	GAG Glu	AAG Lys	GAA Glu	ACA Thr	ACA Thr	TTC Phe	2791
				905				910						915		
AAC Asn	TCC Ser	CTA Leu	CTT Leu	TGT Cys	CCA Pro	TCA Ser	GGT Gly	GGT Gly	GAG Glu	GTT Val	TCT Ser	GAA Glu	GAA Glu	TTA Leu	TCC Ser	2839
			920				925							930		

FIG. 13A

CTG Leu	AAA Lys	CTG Leu	CCA Pro	CCA Pro	AAT Asn	GTG Val	GTA Val	GAA Glu	GAA Glu	TCT Ser	GCC Ala	CGA Arg	GCT Ala	TCT Ser	GTC Val	2887
		935					940					945				
TCA Ser	GTT Val	TTG Leu	GGA Gly	GAC Asp	ATA Ile	TTA Leu	GGC Gly	TCT Ser	GCC Ala	ATG Met	CAA Gln	AAC Asn	ACA Thr	CAA Gln	AAT Asn	2935
	950					955					960					
CTT Leu	CTC Leu	CAG Gln	ATG Met	CCC Pro	TAT Tyr	GGC Gly	TGT Cys	GGA Gly	GAG Glu	CAG Gln	AAT Asn	ATG Met	GTC Val	CTC Leu	TTT Phe	2983
	965				970					975					980	
GCT Ala	CCT Pro	AAC Asn	ATC Ile	TAT Tyr	GTA Val	CTG Leu	GAT Asp	TAT Tyr	CTA Leu	AAT Asn	GAA Glu	ACA Thr	CAG Gln	CAG Gln	CTT Leu	3031
				985					990					995		
ACT Thr	CCA Pro	GAG Glu	GTC Val	AAG Lys	TCC Ser	AAG Lys	GCC Ala	ATT Ile	GGC Gly	TAT Tyr	CTC Leu	AAC Asn	ACT Thr	GGT Gly	TAC Tyr	3079
		1000					1005						1010			
CAG Gln	AGA Arg	CAG Gln	TTG Leu	AAC Asn	TAC Tyr	AAA Lys	CAC His	TAT Tyr	GAT Asp	GGC Gly	TCC Ser	TAC Tyr	AGC Ser	ACC Thr	TTT Phe	3127
		1015					1020					1025				
GGG Gly	GAG Glu	CGA Arg	TAT Tyr	GGC Gly	AGG Arg	AAC Asn	CAG Gln	GGC Gly	AAC Asn	ACC Thr	TGG Trp	CTC Leu	ACA Thr	GCC Ala	TTT Phe	3175
	1030					1035					1040					
GTT Val	CTG Leu	AAG Lys	ACT Thr	TTT Phe	GCC Ala	CAA Gln	GCT Ala	CGA Arg	GCC Ala	TAC Tyr	ATC Ile	TTC Phe	ATC Ile	GAT Asp	GAA Glu	3223
1045					1050				1055					1060		
GCA Ala	CAC His	ATT Ile	ACC Thr	CAA Gln	GCC Ala	CTC Leu	ATA Ile	TGG Trp	CTC Leu	TCC Ser	CAG Gln	AGG Arg	CAG Gln	AAG Lys	GAC Asp	3271
				1065				1070					1075			
AAT Asn	GGC Gly	TGT Cys	TTC Phe	AGG Arg	AGC Ser	TCT Ser	GGG Gly	TCA Ser	CTG Leu	CTC Leu	AAC Asn	AAT Asn	GCC Ala	ATA Ile	AAG Lys	3319
		1080					1085						1090			
GGA Gly	GGA Gly	GTA Val	GAA Glu	GAT Asp	GAA Glu	GTG Val	ACC Thr	CTC Leu	TCC Ser	GCC Ala	TAT Tyr	ATC Ile	ACC Thr	ATC Ile	GCC Ala	3367
		1095				1100						1105				
CTT Leu	CTG Leu	GAG Glu	ATT Ile	CCT Pro	CTC Leu	ACA Thr	GTC Val	ACT Thr	CAC His	CCT Pro	GTT Val	GTC Val	CGC Arg	AAT Asn	GCC Ala	3415
	1110					1115					1120					
CTG Leu	TTT Phe	TGC Cys	CTG Leu	GAG Glu	TCA Ser	GCC Ala	TGG Trp	AAG Lys	ACA Thr	GCA Ala	CAA Gln	GAA Glu	GGG Gly	GAC Asp	CAT His	3463
1125					1130				1135					1140		
GGC Gly	AGC Ser	CAT His	GTA Val	TAT Tyr	ACC Thr	AAA Lys	GCA Ala	CTG Leu	CTG Leu	GCC Ala	TAT Tyr	GCT Ala	TTT Phe	GCC Ala	CTG Leu	3511
			1145				1150						1155			
GCA Ala	GGT Gly	AAC Asn	CAG Gln	GAC Asp	AAG Lys	AGG Arg	AAG Lys	GAA Glu	GTA Val	CTC Leu	AAG Lys	TCA Ser	CTT Leu	AAT Asn	GAG Glu	3559
		1160					1165					1170				

FIG. 13A

GAA GCT GTG AAG AAA GAC AAC TCT GTC CAT TGG GAG CGC CCT CAG AAA Glu Ala Val Lys Lys Asp Asn Ser Val His Trp Glu Arg Pro Gln Lys 1175 1180 1185	3607
CCC AAG GCA CCA GTG GGG CAT TTT TAC GAA CCC CAG GCT CCC TCT GCT Pro Lys Ala Pro Val Gly His Phe Tyr Glu Pro Gln Ala Pro Ser Ala 1190 1195 1200	3655
GAG GTG GAG ATG ACA TCC TAT GTG CTC CTC GCT TAT CTC ACG GCC CAG Glu Val Glu Met Thr Ser Tyr Val Leu Leu Ala Tyr Leu Thr Ala Gln 1205 1210 1215 1220	3703
CCA GCC CCA ACC TCG GAG GAC CTG ACC TCT GCA ACC AAC ATC GTG AAG Pro Ala Pro Thr Ser Glu Asp Leu Thr Ser Ala Thr Asn Ile Val Lys 1225 1230 1235	3751
TGG ATC ACG AAG CAG CAG AAT GCC CAG GGC GGT TTC TCC TCC ACC CAG Trp Ile Thr Lys Gln Gln Asn Ala Gln Gly Gly Phe Ser Ser Thr Gln 1240 1245 1250	3799
GAC ACA GTG GTG GCT CTC CAT GCT CTG TCC AAA TAT GGA GCC GCC ACA Asp Thr Val Val Ala Leu His Ala Leu Ser Lys Tyr Gly Ala Ala Thr 1255 1260 1265	3847
TTT ACC AGG ACT GGG AAG GCT GCA CAG GTG ACT ATC CAG TCT TCA GGG Phe Thr Arg Thr Gly Lys Ala Ala Gln Val Thr Ile Gln Ser Ser Gly 1270 1275 1280	3895
ACA TTT TCC AGC AAA TTC CAA GTG GAC AAC AAC AAT CGC CTG TTA CTG Thr Phe Ser Ser Lys Phe Gln Val Asp Asn Asn Asn Arg Leu Leu Leu 1285 1290 1295 1300	3943
CAG CAG GTC TCA TTG CCA GAG CTG CCT GGG GAA TAC AGC ATG AAA GTG Gln Gln Val Ser Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys Val 1305 1310 1315	3991
ACA GGA GAA GGA TGT GTC TAC CTC CAG ACC TCC TTG AAA TAC AAT ATT Thr Gly Glu Gly Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn Ile 1320 1325 1330	4039
CTC CCA GAA AAG GAA GAG TTC CCC TTT GCT TTA GGA GTG CAG ACT CTG Leu Pro Glu Lys Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr Leu 1335 1340 1345	4087
CCT CAA ACT TGT GAT GAA CCC AAA GCC CAC ACC AGC TTC CAA ATC TCC Pro Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile Ser 1350 1355 1360	4135
CTA AGT GTC AGT TAC ACA GGG AGC CGC TCT GCC TCC AAC ATG GCG ATC Leu Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile 1365 1370 1375 1380	4183
GTT GAT GTG AAG ATG GTC TCT GGC TTC ATT CCC CTG AAG CCA ACA GTG Val Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val 1385 1390 1395	4231
AAA ATG CTT GAA AGA TCT AAC CAT GTG AGC CGG ACA GAA GTC AGC AGC Lys Met Leu Glu Arg Ser Asn His Val Ser Arg Thr Glu Val Ser Ser 1400 1405 1410	4279

FIG. 13A

AAC CAT GTC TTG ATT TAC CTT GAT AAG GTG TCA AAT CAG ACA CTG AGC	4327
Asn His Val Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln Thr Leu Ser	
1415	1420
1425	
TTG TTC TTC ACG GTT CTG CAA GAT GTC CCA GTA AGA GAT CTC AAA CCA	4375
Leu Phe Phe Thr Val Leu Gln Asp Val Pro Val Arg Asp Leu Lys Pro	
1430	1435
1440	
GCC ATA GTG AAA GTC TAT GAT TAC TAC GAG ACG GAT GAG TTT GCA ATC	4423
Ala Ile Val Lys Val Tyr Asp Tyr Tyr Glu Thr Asp Glu Phe Ala Ile	
1445	1450
1455	1460
GCT GAG TAC AAT GCT CCT TGC AGC AAA GAT CTT GGA AAT GCT TGAAGACCA	4474
Ala Glu Tyr Asn Ala Pro Cys Ser Lys Asp Leu Gly Asn Ala	
1465	1470
1	
CAAGGCTGAA AAGTGCTTTG CTGGAGTCCT GTTCTCTGAG CTCCACAGAA GACACGTGTT	4534
TTTGATCTT TAAAGACTTG ATGAATAAAC ACTTTTTCTG GTC	4577

FIG. 13A

Ser Val Ser Gly Lys Pro Gln Tyr Met Val Leu Val Pro Ser Leu Leu  
 1 5 10 15  
 His Thr Glu Thr Thr Glu Lys Gly Cys Val Leu Leu Ser Tyr Leu Asn  
 20 25 30  
 Glu Thr Val Thr Val Ser Ala Ser Leu Glu Ser Val Arg Gly Asn Arg  
 35 40 45  
 Ser Leu Phe Thr Asp Leu Glu Ala Glu Asn Asp Val Leu His Cys Val  
 50 55 60  
 Ala Phe Ala Val Pro Lys Ser Ser Ser Asn Glu Glu Val Met Phe Leu  
 65 70 75 80  
 Thr Val Gln Val Lys Gly Pro Thr Gln Glu Phe Lys Lys Arg Thr Thr  
 85 90 95  
 Val Met Val Lys Asn Glu Asp Ser Leu Val Phe Val Gln Thr Asp Lys  
 100 105 110  
 Ser Ile Tyr Lys Pro Gly Gln Thr Val Lys Phe Arg Val Val Ser Met  
 115 120 125  
 Asp Glu Asn Phe His Pro Leu Asn Glu Leu Ile Pro Leu Val Tyr Ile  
 130 135 140  
 Gln Asp Pro Lys Gly Asn Arg Ile Ala Gln Trp Gln Ser Phe Gln Leu  
 145 150 155 160  
 Glu Gly Gly Leu Lys Gln Phe Ser Phe Pro Leu Ser Ser Glu Pro Phe  
 165 170 175  
 Gln Gly Ser Tyr Lys Val Val Val Gln Lys Lys Ser Gly Gly Arg Thr  
 180 185 190  
 Glu His Pro Phe Thr Val Glu Glu Phe Val Leu Pro Lys Phe Glu Val  
 195 200 205  
 Gln Val Thr Val Pro Lys Ile Ile Thr Ile Leu Glu Glu Glu Met Asn  
 210 215 220  
 Val Ser Val Cys Gly Leu Tyr Thr Tyr Gly Lys Pro Val Pro Gly His  
 225 230 235 240  
 Val Thr Val Ser Ile Cys Arg Lys Tyr Ser Asp Ala Ser Asp Cys His  
 245 250 255  
 Gly Glu Asp Ser Gln Ala Phe Cys Glu Lys Phe Ser Gly Gln Leu Asn  
 260 265 270  
 Ser His Gly Cys Phe Tyr Gln Gln Val Lys Thr Lys Val Phe Gln Leu  
 275 280 285  
 Lys Arg Lys Glu Tyr Glu Met Lys Leu His Thr Glu Ala Gln Ile Gln  
 290 295 300  
 Glu Glu Gly Thr Val Val Glu Leu Thr Gly Arg Gln Ser Ser Glu Ile  
 305 310 315 320  
 Thr Arg Thr Ile Thr Lys Leu Ser Phe Val Lys Val Asp Ser His Phe  
 325 330 335  
 Arg Gln Gly Ile Pro Phe Phe Gly Gln Val Arg Leu Val Asp Gly Lys  
 340 345 350  
 Gly Val Pro Ile Pro Asn Lys Val Ile Phe Ile Arg Gly Asn Glu Ala  
 355 360 365  
 Asn Tyr Tyr Ser Asn Ala Thr Thr Asp Glu His Gly Leu Val Gln Phe  
 370 375 380  
 Ser Ile Asn Thr Thr Asn Val Met Gly Thr Ser Leu Thr Val Arg Val  
 385 390 395 400  
 Asn Tyr Lys Asp Arg Ser Pro Cys Tyr Gly Tyr Gln Trp Val Ser Glu  
 405 410 415  
 Glu His Glu Glu Ala His His Thr Ala Tyr Leu Val Phe Ser Pro Ser  
 420 425 430  
 Lys Ser Phe Val His Leu Glu Pro Met Ser His Glu Leu Pro Cys Gly  
 435 440 445  
 His Thr Gln Thr Val Gln Ala His Tyr Ile Leu Asn Gly Gly Thr Leu  
 450 455 460  
 Leu Gly Leu Lys Lys Leu Ser Phe Tyr Tyr Leu Ile Met Ala Lys Gly

FIG. 13B

465					470						475				480
Gly	Ile	Val	Arg	Thr	Gly	Thr	His	Gly	Leu	Leu	Val	Lys	Gln	Glu	Asp
				485					490						495
Met	Lys	Gly	His	Phe	Ser	Ile	Ser	Ile	Pro	Val	Lys	Ser	Asp	Ile	Ala
			500					505					510		
Pro	Val	Ala	Arg	Leu	Leu	Ile	Tyr	Ala	Val	Leu	Pro	Thr	Gly	Asp	Val
		515					520					525			
Ile	Gly	Asp	Ser	Ala	Lys	Tyr	Asp	Val	Glu	Asn	Cys	Leu	Ala	Asn	Lys
	530					535				540					
Val	Asp	Leu	Ser	Phe	Ser	Pro	Ser	Gln	Ser	Leu	Pro	Ala	Ser	His	Ala
545					550					555					560
His	Leu	Arg	Val	Thr	Ala	Ala	Pro	Gln	Ser	Val	Cys	Ala	Leu	Arg	Ala
				565					570						575
Val	Asp	Gln	Ser	Val	Leu	Leu	Met	Lys	Pro	Asp	Ala	Glu	Leu	Ser	Ala
			580					585					590		
Ser	Ser	Val	Tyr	Asn	Leu	Leu	Pro	Glu	Lys	Asp	Leu	Thr	Gly	Phe	Pro
		595					600					605			
Gly	Pro	Leu	Asn	Asp	Gln	Asp	Asp	Glu	Asp	Cys	Ile	Asn	Arg	His	Asn
	610					615					620				
Val	Tyr	Ile	Asn	Gly	Ile	Thr	Tyr	Thr	Pro	Val	Ser	Ser	Thr	Asn	Glu
625					630					635					640
Lys	Asp	Met	Tyr	Ser	Phe	Leu	Glu	Asp	Met	Gly	Leu	Lys	Ala	Phe	Thr
				645					650						655
Asn	Ser	Lys	Ile	Arg	Lys	Pro	Lys	Met	Cys	Pro	Gln	Leu	Gln	Gln	Tyr
			660					665							670
Glu	Met	His	Gly	Pro	Glu	Gly	Leu	Arg	Val	Gly	Phe	Tyr	Glu	Ser	Asp
		675					680					685			
Val	Met	Gly	Arg	Gly	His	Ala	Arg	Leu	Val	His	Val	Glu	Glu	Pro	His
	690					695					700				
Thr	Glu	Thr	Val	Arg	Lys	Tyr	Phe	Pro	Glu	Thr	Trp	Ile	Trp	Asp	Leu
705					710					715					720
Val	Val	Val	Asn	Ser	Ala	Gly	Val	Ala	Glu	Val	Gly	Val	Thr	Val	Pro
				725					730						735
Asp	Thr	Ile	Thr	Glu	Trp	Lys	Ala	Gly	Ala	Phe	Cys	Leu	Ser	Glu	Asp
			740					745							750
Ala	Gly	Leu	Gly	Ile	Ser	Ser	Thr	Ala	Ser	Leu	Arg	Ala	Phe	Gln	Pro
		755					760					765			
Phe	Phe	Val	Glu	Leu	Thr	Met	Pro	Tyr	Ser	Val	Ile	Arg	Gly	Glu	Ala
	770					775						780			
Phe	Thr	Leu	Lys	Ala	Thr	Val	Leu	Asn	Tyr	Leu	Pro	Lys	Cys	Ile	Arg
785					790					795					800
Val	Ser	Val	Gln	Leu	Ala	Ser	Pro	Ala	Phe	Leu	Ala	Val	Pro	Val	
				805				810							
Glu	Lys	Glu	Gln	Ala	Pro	His	Cys	Ile	Cys	Ala	Asn	Gly	Arg	Gln	Thr
			820					825					830		
Val	Ser	Trp	Ala	Val	Thr	Pro	Lys	Ser	Leu	Gly	Asn	Val	Asn	Phe	Thr
		835					840						845		
Val	Ser	Ala	Glu	Ala	Leu	Glu	Ser	Gln	Glu	Leu	Cys	Gly	Thr	Glu	Val
		850				855					860				
Pro	Ser	Val	Pro	Glu	His	Gly	Arg	Lys	Asp	Thr	Val	Ile	Lys	Pro	Leu
865					870					875					880
Leu	Val	Glu	Pro	Glu	Gly	Leu	Glu	Lys	Glu	Thr	Thr	Phe	Asn	Ser	Leu
				885					890						895
Leu	Cys	Pro	Ser	Gly	Gly	Glu	Val	Ser	Glu	Glu	Leu	Ser	Leu	Lys	Leu
			900					905							910
Pro	Pro	Asn	Val	Val	Glu	Glu	Ser	Ala	Arg	Ala	Ser	Val	Ser	Val	Leu
		915					920								
Gly	Asp	Ile	Leu	Gly	Ser	Ala	Met	Gln	Asn	Thr	Gln	Asn	Leu	Leu	Gln
	930					935									

FIG. 13B

Met Pro Tyr Gly Cys Gly Glu Gln Asn Met Val Leu Phe Ala Pro Asn  
945 950 955 960  
Ile Tyr Val Leu Asp Tyr Leu Asn Glu Thr Gln Gln Leu Thr Pro Glu  
965 970 975  
Val Lys Ser Lys Ala Ile Gly Tyr Leu Asn Thr Gly Tyr Gln Arg Gln  
980 985 990  
Leu Asn Tyr Lys His Tyr Asp Gly Ser Tyr Ser Thr Phe Gly Glu Arg  
995 1000 1005  
Tyr Gly Arg Asn Gln Gly Asn Thr Trp Leu Thr Ala Phe Val Leu Lys  
1010 1015 1020  
Thr Phe Ala Gln Ala Arg Ala Tyr Ile Phe Ile Asp Glu Ala His Ile  
025 1030 1035 1040  
Thr Gln Ala Leu Ile Trp Leu Ser Gln Arg Gln Lys Asp Asn Gly Cys  
1045 1050 1055  
Phe Arg Ser Ser Gly Ser Leu Leu Asn Asn Ala Ile Lys Gly Gly Val  
1060 1065 1070  
Glu Asp Glu Val Thr Leu Ser Ala Tyr Ile Thr Ile Ala Leu Leu Glu  
1075 1080 1085  
Ile Pro Leu Thr Val Thr His Pro Val Val Arg Asn Ala Leu Phe Cys  
1090 1095 1100  
Leu Glu Ser Ala Trp Lys Thr Ala Gln Glu Gly Asp His Gly Ser His  
105 1110 1115 1120  
Val Tyr Thr Lys Ala Leu Leu Ala Tyr Ala Phe Ala Leu Ala Gly Asn  
1125 1130 1135  
Gln Asp Lys Arg Lys Glu Val Leu Lys Ser Leu Asn Glu Glu Ala Val  
1140 1145 1150  
Lys Lys Asp Asn Ser Val His Trp Glu Arg Pro Gln Lys Pro Lys Ala  
1155 1160 1165  
Pro Val Gly His Phe Tyr Glu Pro Gln Ala Pro Ser Ala Glu Val Glu  
1170 1175 1180  
Met Thr Ser Tyr Val Leu Leu Ala Tyr Leu Thr Ala Gln Pro Ala Pro  
185 1190 1195 1200  
Thr Ser Glu Asp Leu Thr Ser Ala Thr Asn Ile Val Lys Trp Ile Thr  
1205 1210 1215  
Lys Gln Gln Asn Ala Gln Gly Gly Phe Ser Ser Thr Gln Asp Thr Val  
1220 1225 1230  
Val Ala Leu His Ala Leu Ser Lys Tyr Gly Ala Ala Thr Phe Thr Arg  
1235 1240 1245  
Thr Gly Lys Ala Ala Gln Val Thr Ile Gln Ser Ser Gly Thr Phe Ser  
1250 1255 1260  
Ser Lys Phe Gln Val Asp Asn Asn Asn Arg Leu Leu Leu Gln Gln Val  
265 1270 1275 1280  
Ser Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys Val Thr Gly Glu  
1285 1290 1295  
Gly Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn Ile Leu Pro Glu  
1300 1305 1310  
~~Lys Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr Leu Pro Gln Thr~~  
1315 1320 1325  
~~Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile Ser Leu Ser Val~~  
1330 1335 1340  
~~Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile Val Asp Val~~  
345 1350 1355 1360  
~~Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val Lys Met Leu~~  
1365 1370 1375  
~~Glu Arg Ser Asn His Val Ser Arg Thr Glu Val Ser Ser Asn His Val~~  
1380 1385 1390  
~~Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln Thr Leu Ser Leu Phe Phe~~  
1395 1400 1405

FIG. 13B

Thr Val Leu Gln Asp Val Pro Val Arg Asp Leu Lys Pro Ala Ile Val  
1410 1415 1420  
Lys Val Tyr Asp Tyr Tyr Glu Thr Asp Glu Phe Ala Ile Ala Glu Tyr  
425 1430 1435 1440  
Asn Ala Pro Cys Ser Lys Asp Leu Gly Asn Ala  
1445 1450

FIG. 13B

CAGCGGTGCG	AGCTCCAGGC	CCATGCACTG	AGGAGGCGGA	AAACAAGGGGA	GCCCCCAGAG	60
CTCCATCAAG	CCCCCTCCAA	AGGCTCCCCT	ACCCGGTCCA	CGCCCCCCAC	CCCCCTCCC	120
CGCCTCCTCC	CAATTGTGCA	TTTTTGACG	CGGAGGCGGC	TCCGAGATGG	GGCTGTGAGC	180
TTCGCCCCGG	GAGGGGGAAA	GAGCAGCGAG	GAGTGAAGCG	GGGGGGTGGG	GTGAAGGGTT	240
TGGATTTCCG	GGCAGGGGGC	GCACCCCGT	CAGCAGGCC	TCCCCAAGGG	GCTCGGAACT	300
CTACCTCTTC	ACCCACGCC	CTGGTGCCT	TTGCCGAAGG	AAAGAATAAG	AACAGAGAAG	360
GAGGAGGGGG	AAAGGAGGAA	AAGGGGGACC	CCCCAACTGG	GGGGGGTGAA	GGAGAGAAGT	420
AGCAGGACCA	GAGGGGAAGG	GGCTGCTGCT	TGCATCAGCC	CACACC	ATG CTG ACC	475
					Met Leu Thr	
					1	
CCG CCG TTG CTC CTG CTG CTG CCC CTG CTC TCA GCT CTG GTC GCG GCG						523
Pro Pro Leu Leu Leu Leu Leu Pro Leu Leu Ser Ala Leu Val Ala Ala	5		10		15	
GCT ATC GAC GCC CCT AAG ACT TGC AGC CCC AAG CAG TTT GCC TGC AGA						571
Ala Ile Asp Ala Pro Lys Thr Cys Ser Pro Lys Gln Phe Ala Cys Arg	20	25	30		35	
GAT CAA ATA ACC TGT ATC TCA AAG GGC TGG CGG TGC GAC GGT GAG AGG						619
Asp Gln Ile Thr Cys Ile Ser Lys Gly Trp Arg Cys Asp Gly Glu Arg		40		45	50	
GAC TGC CCA GAC GGA TCT GAC GAG GCC CCT GAG ATT TGT CCA CAG AGT						667
Asp Cys Pro Asp Gly Ser Asp Glu Ala Pro Glu Ile Cys Pro Gln Ser		55		60	65	
AAG GCC CAG CGA TGC CAG CCA AAC GAG CAT AAC TGC CTG GGT ACT GAG						715
Lys Ala Gln Arg Cys Gln Pro Asn Glu His Asn Cys Leu Gly Thr Glu		70		75	80	
CTG TGT GTT CCC ATG TCC CGC CTC TGC AAT GGG GTC CAG GAC TGC ATG						763
Leu Cys Val Pro Met Ser Arg Leu Cys Asn Gly Val Gln Asp Cys Met		85		90	95	
GAC GGC TCA GAT GAG GGG CCC CAC TGC CGA GAG CTC CAA GGC AAC TGC						811
Asp Gly Ser Asp Glu Gly Pro His Cys Arg Glu Leu Gln Gly Asn Cys	100		105		110	115
TCT CGC CTG GGC TGC CAG CAC CAT TGT GTC CCC ACA CTC GAT GGG CCC						859
Ser Arg Leu Gly Cys Gln His His Cys Val Pro Thr Leu Asp Gly Pro		120		125	130	
ACC TGC TAC TGC AAC AGC AGC TTT CAG CTT CAG GCA GAT GGC AAG ACC						907
Thr Cys Tyr Cys Asn Ser Ser Phe Gln Leu Gln Ala Asp Gly Lys Thr		135		140	145	
TGC AAA GAT TTT GAT GAG TGC TCA GTG TAC GGC ACC TGC AGC CAG CTA						955
Cys Lys Asp Phe Asp Glu Cys Ser Val Tyr Gly Thr Cys Ser Gln Leu		150		155	160	
TGC ACC AAC ACA GAC GGC TCC TTC ATA TGT GGC TGT GTT GAA GGA TAC						1003
Cys Thr Asn Thr Asp Gly Ser Phe Ile Cys Gly Cys Val Glu Gly Tyr		165		170	175	
CTC CTG CAG CCG GAT AAC CGC TCC TGC AAG GCC AAG AAC GAG CCA GTA						1051
Leu Leu Gln Pro Asp Asn Arg Ser Cys Lys Ala Lys Asn Glu Pro Val	180		185		190	195

FIG. 14A

GAC CGG CCC CCT GTG CTG TTG ATA GCC AAC TCC CAG AAC ATC TTG GCC Asp Arg Pro Pro Val Leu Leu Ile Ala Asn Ser Gln Asn Ile Leu Ala 200 205 210	1099
ACG TAC CTG AGT GGG GCC CAG GTG TCT ACC ATC ACA CCT ACG AGC ACG Thr Tyr Leu Ser Gly Ala Gln Val Ser Thr Ile Thr Pro Thr Ser Thr 215 220 225	1147
CGG CAG ACC ACA GCC ATG GAC TTC AGC TAT GCC AAC GAG ACC GTA TGC Arg Gln Thr Thr Ala Met Asp Phe Ser Tyr Ala Asn Glu Thr Val Cys 230 235 240	1195
TGG GTG CAT GTT GGG GAC AGT GCT GCT CAG ACG CAG CTC AAG TGT GCC Trp Val His Val Gly Asp Ser Ala Ala Gln Thr Gln Leu Lys Cys Ala 245 250 255	1243
CGC ATG CCT GGC CTA AAG GGC TTC GTG GAT GAG CAC ACC ATC AAC ATC Arg Met Pro Gly Leu Lys Gly Phe Val Asp Glu His Thr Ile Asn Ile 260 265 270 275	1291
TCC CTC AGT CTG CAC CAC GTG GAA CAG ATG GCC ATC GAC TGG CTG ACA Ser Leu Ser Leu His His Val Glu Gln Met Ala Ile Asp Trp Leu Thr 280 285 290	1339
GGC AAC TTC TAC TTT GTG GAT GAC ATC GAT GAT AGG ATC TTT GTC TGC Gly Asn Phe Tyr Phe Val Asp Asp Ile Asp Asp Arg Ile Phe Val Cys 295 300 305	1387
AAC AGA AAT GGG GAC ACA TGT GTC ACA TTG CTA GAC CTG GAA CTC TAC Asn Arg Asn Gly Asp Thr Cys Val Thr Leu Leu Asp Leu Glu Leu Tyr 310 315 320	1435
AAC CCC AAG GGC ATT GCC CTG GAC CCT GCC ATG GGG AAG GTG TTT TTC Asn Pro Lys Gly Ile Ala Leu Asp Pro Ala Met Gly Lys Val Phe Phe 325 330 335	1483
ACT GAC TAT GGG CAG ATC CCA AAG GTG GAA CGC TGT GAC ATG GAT GGG Thr Asp Tyr Gly Gln Ile Pro Lys Val Glu Arg Cys Asp Met Asp Gly 340 345 350 355	1531
CAG AAC CGC ACC AAG CTC GTC GAC AGC AAG ATT GTG TTT CCT CAT GGC Gln Asn Arg Thr Lys Leu Val Asp Ser Lys Ile Val Phe Pro His Gly 360 365 370	1579
ATC ACG CTG GAC CTG GTC AGC CGC CTT GTC TAC TGG GCA GAT GCC TAT Ile Thr Leu Asp Leu Val Ser Arg Leu Val Tyr Trp Ala Asp Ala Tyr 375 380 385	1627
CTG GAC TAT ATT GAA GTG GTG GAC TAT GAG GGC AAG GGC CGC CAG ACC Leu Asp Tyr Ile Glu Val Val Asp Tyr Glu Gly Lys Gly Arg Gln Thr 390 395 400	1675
ATC ATC CAG GGC ATC CTG ATT GAG CAC CTG TAC GGC CTG ACT GTG TTT Ile Ile Gln Gly Ile Leu Ile Glu His Leu Tyr Gly Leu Thr Val Phe 405 410 415	1723
GAG AAT TAT CTC TAT GCC ACC AAC TCG GAC AAT GCC AAT GCC CAG CAG Glu Asn Tyr Leu Tyr Ala Thr Asn Ser Asp Asn Ala Asn Ala Gln Gln 420 425 430 435	1771

FIG. 14A

AAG Lys	ACG Thr	AGT Ser	GTG Val	ATC Ile	CGT Arg	GTG Val	AAC Asn	CGC Arg	TTT Phe	AAC Asn	AGC Ser	ACC Thr	GAG Glu	TAC Tyr	CAG Gln	1819
			440						445					450		
GTT Val	GTC Val	ACC Thr	CGG Arg	GTG Val	GAC Asp	AAG Lys	GGT Gly	GGT Gly	GCC Ala	CTC Leu	CAC His	ATC Ile	TAC Tyr	CAC His	CAG Gln	1867
			455						460					465		
AGG Arg	CGT Arg	CAG Gln	CCC Pro	CGA Arg	GTG Val	AGG Arg	AGC Ser	CAT His	GCC Ala	TGT Cys	GAA Glu	AAC Asn	GAC Asp	CAG Gln	TAT Tyr	1915
			470						475					480		
GGG Gly	AAG Lys	CCG Pro	GGT Gly	GGC Gly	TGC Cys	TCT Ser	GAC Asp	ATC Ile	TGC Cys	CTG Leu	CTG Leu	GCC Ala	AAC Asn	AGC Ser	CAC His	1963
			485						490					495		
AAG Lys	GCG Ala	CGG Arg	ACC Thr	TGC Cys	CGC Arg	TGC Cys	CGT Arg	TCC Ser	GGC Gly	TTC Phe	AGC Ser	CTG Leu	GGC Gly	AGT Ser	GAC Asp	2011
			500						505					510		515
GGG Gly	AAG Lys	TCA Ser	TGC Cys	AAG Lys	AAG Lys	CCG Pro	GAG Glu	CAT His	GAG Glu	CTG Leu	TTC Phe	CTC Leu	GTG Val	TAT Tyr	GGC Gly	2059
			520						525					530		
AAG Lys	GGC Gly	CGG Arg	CCA Pro	GGC Gly	ATC Ile	ATC Ile	CGG Arg	GGC Gly	ATG Met	GAT Asp	ATG Met	GGG Gly	GCC Ala	AAG Lys	GTC Val	2107
			535						540					545		
CCG Pro	GAT Asp	GAG Glu	CAC His	ATG Met	ATC Ile	CCC Pro	ATT Ile	GAA Glu	AAC Asn	CTC Leu	ATG Met	AAC Asn	CCC Pro	CGA Arg	GCC Ala	2155
			550						555					560		
CTG Leu	GAC Asp	TTC Phe	CAC His	GCT Ala	GAG Glu	ACC Thr	GGC Gly	TTC Phe	ATC Ile	TAC Tyr	TTT Phe	GCC Ala	GAC Asp	ACC Thr	ACC Thr	2203
			565						570					575		
AGC Ser	TAC Tyr	CTC Leu	ATT Ile	GGC Gly	CGC Arg	CAG Gln	AAG Lys	ATT Ile	GAT Asp	GGC Gly	ACT Thr	GAG Glu	CGG Arg	GAG Glu	ACC Thr	2251
			580						585					590		595
ATC Ile	CTG Leu	AAG Lys	GAC Asp	GGC Gly	ATC Ile	CAC His	AAT Asn	GTG Val	GAG Glu	GGT Gly	GTG Val	GCC Ala	GTG Val	GAC Asp	TGG Trp	2299
			600						605					610		
ATG Met	GGA Gly	GAC Asp	AAT Asn	CTG Leu	TAC Tyr	TGG Trp	ACG Thr	GAC Asp	GAT Asp	GGG Gly	CCC Pro	AAA Lys	AAG Lys	ACA Thr	ATC Ile	2347
			615						620					625		
AGC Ser	GTG Val	GCC Ala	AGG Arg	CTG Leu	GAG Glu	AAA Lys	GCT Ala	GCT Ala	CAG Gln	ACC Thr	CGC Arg	AAG Lys	ACT Thr	TTA Leu	ATC Ile	2395
			630						635					640		
GAG Glu	GGC Gly	AAA Lys	ATG Met	ACA Thr	CAC His	CCC Pro	AGG Arg	GCT Ala	ATT Ile	GTG Val	GTG Val	GAT Asp	CCA Pro	CTC Leu	AAT Asn	2443
			645						650					655		
GGG Gly	TGG Trp	ATG Met	TAC Tyr	TGG Trp	ACA Thr	GAC Asp	TGG Trp	GAG Glu	GAG Glu	GAC Asp	CCC Pro	AAG Lys	GAC Asp	AGT Ser	CGG Arg	2491
			660						665					670		675

FIG. 14A

CGT	GGG	CGG	CTG	GAG	AGG	GCG	TGG	ATG	GAT	GGC	TCA	CAC	CGA	GAC	ATC	2539
Arg	Gly	Arg	Leu	Glu	Arg	Ala	Trp	Met	Asp	Gly	Ser	His	Arg	Asp	Ile	
				680					685					690		
TTT	GTC	ACC	TCC	AAG	ACA	GTG	CTT	TGG	CCC	AAT	GGG	CTA	AGC	CTG	GAC	2587
Phe	Val	Thr	Ser	Lys	Thr	Val	Leu	Trp	Pro	Asn	Gly	Leu	Ser	Leu	Asp	
			695					700					705			
ATC	CCG	GCT	GGG	CGC	CTC	TAC	TGG	GTG	GAT	GCC	TTC	TAC	GAC	CGC	ATC	2635
Ile	Pro	Ala	Gly	Arg	Leu	Tyr	Trp	Val	Asp	Ala	Phe	Tyr	Asp	Arg	Ile	
		710					715					720				
GAG	ACG	ATA	CTG	CTC	AAT	GGC	ACA	GAC	CGG	AAG	ATT	GTG	TAT	GAA	GGT	2683
Glu	Thr	Ile	Leu	Leu	Asn	Gly	Thr	Asp	Arg	Lys	Ile	Val	Tyr	Glu	Gly	
	725					730					735					
CCT	GAG	CTG	AAC	CAC	GCC	TTT	GGC	CTG	TGT	CAC	CAT	GGC	AAC	TAC	CTC	2731
Pro	Glu	Leu	Asn	His	Ala	Phe	Gly	Leu	Cys	His	His	Gly	Asn	Tyr	Leu	
740					745					750					755	
TTC	TGG	ACT	GAG	TAT	CGG	AGT	GGC	AGT	GTC	TAC	CGC	TTG	GAA	CGG	GGT	2779
Phe	Trp	Thr	Glu	Tyr	Arg	Ser	Gly	Ser	Val	Tyr	Arg	Leu	Glu	Arg	Gly	
			760					765						770		
GTA	GGA	GGC	GCA	CCC	CCC	ACT	GTG	ACC	CTT	CTG	CGC	AGT	GAG	CGG	CCC	2827
Val	Gly	Gly	Ala	Pro	Pro	Thr	Val	Thr	Leu	Leu	Arg	Ser	Glu	Arg	Pro	
			775					780					785			
CCC	ATC	TTT	GAG	ATC	CGA	ATG	TAT	GAT	GCC	CAG	CAG	CAG	CAA	GTT	GGC	2875
Pro	Ile	Phe	Glu	Ile	Arg	Met	Tyr	Asp	Ala	Gln	Gln	Gln	Gln	Val	Gly	
		790					795					800				
ACC	AAC	AAA	TGC	CGG	GTG	AAC	AAT	GGC	GGC	TGC	AGC	AGC	CTG	TGC	TTG	2923
Thr	Asn	Lys	Cys	Arg	Val	Asn	Asn	Gly	Gly	Cys	Ser	Ser	Leu	Cys	Leu	
	805					810					815					
GCC	ACC	CCT	GGG	AGC	CGC	CAG	TGC	GCC	TGT	GCT	GAG	GAC	CAG	GTG	TTG	2971
Ala	Thr	Pro	Gly	Ser	Arg	Gln	Cys	Ala	Cys	Ala	Glu	Asp	Gln	Val	Leu	
820					825				830						835	
GAC	GCA	GAC	GGC	GTC	ACT	TGC	TTG	GCG	AAC	CCA	TCC	TAC	GTG	CCT	CCA	3019
Asp	Ala	Asp	Gly	Val	Thr	Cys	Leu	Ala	Asn	Pro	Ser	Tyr	Val	Pro	Pro	
				840					845					850		
CCC	CAG	TGC	CAG	CCA	GGC	GAG	TTT	GCC	TGT	GCC	AAC	AGC	CGC	TGC	ATC	3067
Pro	Gln	Cys	Gln	Pro	Gly	Glu	Phe	Ala	Cys	Ala	Asn	Ser	Arg	Cys	Ile	
			855					860					865			
CAG	GAG	CGC	TGG	AAG	TGT	GAC	GGA	GAC	AAC	GAT	TGC	CTG	GAC	AAC	AGT	3115
Gln	Glu	Arg	Trp	Lys	Cys	Asp	Gly	Asp	Asn	Asp	Cys	Leu	Asp	Asn	Ser	
		870					875					880				
GAT	GAG	GCC	CCA	GCC	CTC	TGC	CAT	CAG	CAC	ACC	TGC	CCC	TCG	GAC	CGA	3163
Asp	Glu	Ala	Pro	Ala	Leu	Cys	His	Gln	His	Thr	Cys	Pro	Ser	Asp	Arg	
	885					890					895					
TTC	AAG	TGC	GAG	AAC	AAC	CGG	TGC	ATC	CCC	AAC	CGC	TGG	CTC	TGC	GAC	3211
Phe	Lys	Cys	Glu	Asn	Asn	Arg	Cys	Ile	Pro	Asn	Arg	Trp	Leu	Cys	Asp	
900				905						910					915	

FIG. 14A

GGG GAC AAT GAC TGT GGG AAC AGT GAA GAT GAG TCC AAT GCC ACT TGT Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys 920 925 930	3259
TCA GCC CGC ACC TGC CCC CCC AAC CAG TTC TCC TGT GCC AGT GGC CGC Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg 935 940 945	3307
TGC ATC CCC ATC TCC TGG ACG TGT GAT CTG GAT GAC GAC TGT GGG GAC Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp Asp Cys Gly Asp 950 955 960	3355
CGC TCT GAT GAG TCT GCT TCG TGT GCC TAT CCC ACC TGC TTC CCC CTG Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu 965 970 975	3403
ACT CAG TTT ACC TGC AAC AAT GGC AGA TGT ATC AAC ATC AAC TGG AGA Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg 980 985 990	3451
TGC GAC AAT GAC AAT GAC TGT GGG GAC AAC AGT GAC GAA GCC GGC TGC Cys Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Ala Gly Cys 1000 1005 1010	3499
AGC CAC TCC TGT TCT AGC ACC CAG TTC AAG TGC AAC AGC GGG CGT TGC Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys Asn Ser Gly Arg Cys 1015 1020 1025	3547
ATC CCC GAG CAC TGG ACC TGC GAT GGG GAC AAT GAC TGC GGA GAC TAC Ile Pro Glu His Trp Thr Cys Asp Gly Asp Asn Asp Cys Gly Asp Tyr 1030 1035 1040	3595
AGT GAT GAG ACA CAC GCC AAC TGC ACC AAC CAG GCC ACG AGG CCC CCT Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln Ala Thr Arg Pro Pro 1045 1050 1055	3643
GGT GGC TGC CAC ACT GAT GAG TTC CAG TGC CGG CTG GAT GGA CTA TGC Gly Gly Cys His Thr Asp Glu Phe Gln Cys Arg Leu Asp Gly Leu Cys 1060 1065 1070 1075	3691
ATC CCC CTG CGG TGG CGC TGC GAT GGG GAC ACT GAC TGC ATG GAC TCC Ile Pro Leu Arg Trp Arg Cys Asp Gly Asp Thr Asp Cys Met Asp Ser 1080 1085 1090	3739
AGC GAT GAG AAG AGC TGT GAG GGA GTG ACC CAC GTC TGC GAT CCC AGT Ser Asp Glu Lys Ser Cys Glu Gly Val Thr His Val Cys Asp Pro Ser 1095 1100 1105	3787
GTC AAG TTT GGC TGC AAG GAC TCA GCT CGG TGC ATC AGC AAA GCG TGG Val Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys Ile Ser Lys Ala Trp 1110 1115 1120	3835
GTG TGT GAT GGC GAC AAT GAC TGT GAG GAT AAC TCG GAC GAG GAG AAC Val Cys Asp Gly Asp Asn Asp Cys Glu Asp Asn Ser Asp Glu Glu Asn 1125 1130 1135	3883
TGC GAG TCC CTG GCC TGC AGG CCA CCC TCG CAC CCT TGT GCC AAC AAC Cys Glu Ser Leu Ala Cys Arg Pro Pro Ser His Pro Cys Ala Asn Asn 1140 1145 1150 1155	3931

FIG. 14A

ACC	TCA	GTC	TGC	CTG	CCC	CCT	GAC	AAG	CTG	TGT	GAT	GGC	AAC	GAC	GAC	3979
Thr	Ser	Val	Cys	Leu	Pro	Pro	Asp	Lys	Leu	Cys	Asp	Gly	Asn	Asp	Asp	
				1160					1165						1170	
TGT	GGC	GAC	GGC	TCA	GAT	GAG	GGC	GAG	CTC	TGC	GAC	CAG	TGC	TCT	CTG	4027
Cys	Gly	Asp	Gly	Ser	Asp	Glu	Gly	Glu	Leu	Cys	Asp	Gln	Cys	Ser	Leu	
			1175					1180							1185	
AAT	AAC	GGT	GGC	TGC	AGC	CAC	AAC	TGC	TCA	GTG	GCA	CCT	GGC	GAA	GGC	4075
Asn	Asn	Gly	Gly	Cys	Ser	His	Asn	Cys	Ser	Val	Ala	Pro	Gly	Glu	Gly	
		1190						1195					1200			
ATT	GTG	TGT	TCC	TGC	CCT	CTG	GGC	ATG	GAG	CTG	GGG	CCC	GAC	AAC	CAC	4123
Ile	Val	Cys	Ser	Cys	Pro	Leu	Gly	Met	Glu	Leu	Gly	Pro	Asp	Asn	His	
	1205					1210					1215					
ACC	TGC	CAG	ATC	CAG	AGC	TAC	TGT	GCC	AAG	CAT	CTC	AAA	TGC	AGC	CAA	4171
Thr	Cys	Gln	Ile	Gln	Ser	Tyr	Cys	Ala	Lys	His	Leu	Lys	Cys	Ser	Gln	
1220					1225					1230					1235	
AAG	TGC	GAC	CAG	AAC	AAG	TTC	AGC	GTG	AAG	TGC	TCC	TGC	TAC	GAG	GGC	4219
Lys	Cys	Asp	Gln	Asn	Lys	Phe	Ser	Val	Lys	Cys	Ser	Cys	Tyr	Glu	Gly	
				1240					1245						1250	
TGG	GTC	CTG	GAA	CCT	GAC	GGC	GAG	AGC	TGC	CGC	AGC	CTG	GAC	CCC	TTC	4267
Trp	Val	Leu	Glu	Pro	Asp	Gly	Glu	Ser	Cys	Arg	Ser	Leu	Asp	Pro	Phe	
			1255					1260						1265		
AAG	CCG	TTC	ATC	ATT	TTC	TCC	AAC	CGC	CAT	GAA	ATC	CGG	CGC	ATC	GAT	4315
Lys	Pro	Phe	Ile	Ile	Phe	Ser	Asn	Arg	His	Glu	Ile	Arg	Arg	Ile	Asp	
		1270					1275					1280				
CTT	CAC	AAA	GGA	GAC	TAC	AGC	GTC	CTG	GTG	CCC	GGC	CTG	CGC	AAC	ACC	4363
Leu	His	Lys	Gly	Asp	Tyr	Ser	Val	Leu	Val	Pro	Gly	Leu	Arg	Asn	Thr	
	1285					1290					1295					
ATC	GCC	CTG	GAC	TTC	CAC	CTC	AGC	CAG	AGC	GCC	CTC	TAC	TGG	ACC	GAC	4411
Ile	Ala	Leu	Asp	Phe	His	Leu	Ser	Gln	Ser	Ala	Leu	Tyr	Trp	Thr	Asp	
1300					1305					1310					1315	
GTG	GTG	GAG	GAC	AAG	ATC	TAC	CGC	GGG	AAG	CTG	CTG	GAC	AAC	GGA	GCC	4459
Val	Val	Glu	Asp	Lys	Ile	Tyr	Arg	Gly	Lys	Leu	Leu	Asp	Asn	Gly	Ala	
				1320					1325						1330	
CTG	ACT	AGT	TTC	GAG	GTG	GTG	ATT	CAG	TAT	GGC	CTG	GCC	ACA	CCC	GAG	4507
Leu	Thr	Ser	Phe	Glu	Val	Val	Ile	Gln	Tyr	Gly	Leu	Ala	Thr	Pro	Glu	
			1335					1340						1345		
GGC	CTG	GCT	GTA	GAC	TGG	ATT	GCA	GGC	AAC	ATC	TAC	TGG	GTG	GAG	AGT	4555
Gly	Leu	Ala	Val	Asp	Trp	Ile	Ala	Gly	Asn	Ile	Tyr	Trp	Val	Glu	Ser	
		1350					1355					1360				
AAC	CTG	GAT	CAG	ATC	GAG	GTG	GCC	AAG	CTG	GAT	GGG	ACC	CTC	CGG	ACC	4603
Asn	Leu	Asp	Gln	Ile	Glu	Val	Ala	Lys	Leu	Asp	Gly	Thr	Leu	Arg	Thr	
	1365					1370					1375					
ACC	CTG	CTG	GCC	GGT	GAC	ATT	GAG	CAC	CCA	AGG	GCA	ATC	GCA	CTG	GAT	4651
Thr	Leu	Leu	Ala	Gly	Asp	Ile	Glu	His	Pro	Arg	Ala	Ile	Ala	Leu	Asp	
1380					1385					1390					1395	

FIG. 14A

CCC CGG GAT GGG ATC CTG TTT TGG ACA GAC TGG GAT GCC AGC CTG CCC Pro Arg Asp Gly Ile Leu Phe Trp Thr Asp Trp Asp Ala Ser Leu Pro 1400 1405 1410	4699
CGC ATT GAG GCA GCC TCC ATG AGT GGG GCT GGG CGC CGC ACC GTG CAC Arg Ile Glu Ala Ala Ser Met Ser Gly Ala Gly Arg Arg Thr Val His 1415 1420 1425	4747
CGG GAG ACC GGC TCT GGG GGC TGG CCC AAC GGG CTC ACC GTG GAC TAC Arg Glu Thr Gly Ser Gly Gly Trp Pro Asn Gly Leu Thr Val Asp Tyr 1430 1435 1440	4795
CTG GAG AAG CGC ATC CTT TGG ATT GAC GCC AGG TCA GAT GCC ATT TAC Leu Glu Lys Arg Ile Leu Trp Ile Asp Ala Arg Ser Asp Ala Ile Tyr 1445 1450 1455	4843
TCA GCC CGT TAC GAC GGC TCT GGC CAC ATG GAG GTG CTT CGG GGA CAC Ser Ala Arg Tyr Asp Gly Ser Gly His Met Glu Val Leu Arg Gly His 1460 1465 1470 1475	4891
GAG TTC CTG TCG CAC CCG TTT GCA GTG ACG CTG TAC GGG GGG GAG GTC Glu Phe Leu Ser His Pro Phe Ala Val Thr Leu Tyr Gly Gly Glu Val 1480 1485 1490	4939
TAC TGG ACT GAC TGG CGA ACA AAC ACA CTG GCT AAG GCC AAC AAG TGG Tyr Trp Thr Asp Trp Arg Thr Asn Thr Leu Ala Lys Ala Asn Lys Trp 1495 1500 1505	4987
ACC GGC CAC AAT GTC ACC GTG GTA CAG AGG ACC AAC ACC CAG CCC TTT Thr Gly His Asn Val Thr Val Val Gln Arg Thr Asn Thr Gln Pro Phe 1510 1515 1520	5035
GAC CTG CAG GTG TAC CAC CCC TCC CGC CAG CCC ATG GCT CCC AAT CCC Asp Leu Gln Val Tyr His Pro Ser Arg Gln Pro Met Ala Pro Asn Pro 1525 1530 1535	5083
TGT GAG GCC AAT GGG GGC CAG GGC CCC TGC TCC CAC CTG TGT CTC ATC Cys Glu Ala Asn Gly Gly Gln Gly Pro Cys Ser His Leu Cys Leu Ile 1540 1545 1550 1555	5131
AAC TAC AAC CGG ACC GTG TCC TGC GCC TGC CCC CAC CTC ATG AAG CTC Asn Tyr Asn Arg Thr Val Ser Cys Ala Cys Pro His Leu Met Lys Leu 1560 1565 1570	5179
CAC AAG GAC AAC ACC ACC TGC TAT GAG TTT AAG AAG TTC CTG CTG TAC His Lys Asp Asn Thr Thr Cys Tyr Glu Phe Lys Lys Phe Leu Leu Tyr 1575 1580 1585	5227
GCA CGT CAG ATG GAG ATC CGA GGT GTG GAC CTG GAT GCT CCC TAC TAC Ala Arg Gln Met Glu Ile Arg Gly Val Asp Leu Asp Ala Pro Tyr Tyr 1590 1595 1600	5275
AAC TAC ATC ATC TCC TTC ACG GTG CCC GAC ATC GAC AAC GTC ACA GTG Asn Tyr Ile Ile Ser Phe Thr Val Pro Asp Ile Asp Asn Val Thr Val 1605 1610 1615	5323
CTA GAC TAC GAT GCC CGC GAG CAG CGT GTG TAC TGG TCT GAC GTG CGG Leu Asp Tyr Asp Ala Arg Glu Gln Arg Val Tyr Trp Ser Asp Val Arg 1620 1625 1630 1635	5371

FIG. 14A

ACA Thr	CAG Gln	GCC Ala	ATC Ile	AAG Lys	CGG Arg	GCC Ala	TTC Phe	ATC Ile	AAC Asn	GGC Gly	ACA Thr	GGC Gly	GTG Val	GAG Glu	ACA Thr	5419
			1640					1645					1650			
GTC Val	GTC Val	TCT Ser	GCA Ala	GAC Asp	TTG Leu	CCA Pro	AAT Asn	GCC Ala	CAC His	GGG Gly	CTG Leu	GCT Ala	GTG Val	GAC Asp	TGG Trp	5467
			1655					1660					1665			
GTC Val	TCC Ser	CGA Arg	AAC Asn	CTG Leu	TTC Phe	TGG Trp	ACA Thr	AGC Ser	TAT Tyr	GAC Asp	ACC Thr	AAT Asn	AAG Lys	AAG Lys	CAG Gln	5515
			1670					1675					1680			
ATC Ile	AAT Asn	GTG Val	GCC Ala	CGG Arg	CTG Leu	GAT Asp	GGC Gly	TCC Ser	TTC Phe	AAG Lys	AAC Asn	GCA Ala	GTG Val	GTG Val	CAG Gln	5563
			1685					1690					1695			
GGC Gly	CTG Leu	GAG Glu	CAG Gln	CCC Pro	CAT His	GGC Gly	CTT Leu	GTC Val	GTC Val	CAC His	CCT Pro	CTG Leu	CGT Arg	GGG Gly	AAG Lys	5611
1700					1705					1710					1715	
CTC Leu	TAC Tyr	TGG Trp	ACC Thr	GAT Asp	GGT Gly	GAC Asp	AAC Asn	ATC Ile	AGC Ser	ATG Met	GCC Ala	AAC Asn	ATG Met	GAT Asp	GGC Gly	5659
				1720						1725				1730		
AGC Ser	AAT Asn	CGC Arg	ACC Thr	CTG Leu	CTC Leu	TTC Phe	AGT Ser	GGC Gly	CAG Gln	AAG Lys	GGC Gly	CCC Pro	GTG Val	GGC Gly	CTG Leu	5707
				1735				1740					1745			
GCT Ala	ATT Ile	GAC Asp	TTC Phe	CCT Pro	GAA Glu	AGC Ser	AAA Lys	CTC Leu	TAC Tyr	TGG Trp	ATC Ile	AGC Ser	TCC Ser	GGG Gly	AAC Asn	5755
		1750					1755					1760				
CAT His	ACC Thr	ATC Ile	AAC Asn	CGC Arg	TGC Cys	AAC Asn	CTG Leu	GAT Asp	GGG Gly	AGT Ser	GGG Gly	CTG Leu	GAG Glu	GTC Val	ATC Ile	5803
							1770				1775					
GAT Asp	GCC Ala	ATG Met	CGG Arg	AGC Ser	CAG Gln	CTG Leu	GGC Gly	AAG Lys	GCC Ala	ACC Thr	GCC Ala	CTG Leu	GCC Ala	ATC Ile	ATG Met	5851
1780					1785					1790					1795	
GGG Gly	GAC Asp	AAG Lys	CTG Leu	TGG Trp	TGG Trp	GCT Ala	GAT Asp	CAG Gln	GTG Val	TCG Ser	GAA Glu	AAG Lys	ATG Met	GGC Gly	ACA Thr	5899
				1800					1805					1810		
TGC Cys	AGC Ser	AAG Lys	GCT Ala	GAC Asp	GGC Gly	TCG Ser	GGC Gly	TCC Ser	GTG Val	GTC Val	CTT Leu	CGG Arg	AAC Asn	AGC Ser	ACC Thr	5947
			1815					1820					1825			
ACC Thr	CTG Leu	GTG Val	ATG Met	CAC His	ATG Met	AAG Lys	GTC Val	TAT Tyr	GAC Asp	GAG Glu	AGC Ser	ATC Ile	CAG Gln	CTG Leu	GAC Asp	5995
			1830				1835					1840				
CAT His	AAG Lys	GGC Gly	ACC Thr	AAC Asn	CCC Pro	TGC Cys	AGT Ser	GTC Val	AAC Asn	AAC Asn	GGT Gly	GAC Asp	TGC Cys	TCC Ser	CAG Gln	6043
			1845			1850					1855					
CTC Leu	TGC Cys	CTG Leu	CCC Pro	ACG Thr	TCA Ser	GAG Glu	ACG Thr	ACC Thr	CGC Arg	TCC Ser	TGC Cys	ATG Met	TGC Cys	ACA Thr	GCC Ala	6091
1860					1865					1870					1875	

FIG. 14A

GGC TAT AGC CTC CGG AGT GGC CAG CAG GCC TGC GAG GGC GTA GGT TCC Gly Tyr Ser Leu Arg Ser Gly Gln Gln Ala Cys Glu Gly Val Gly Ser 1880 1885 1890	6139
TTT CTC CTG TAC TCT GTG CAT GAG GGA ATC AGG GGA ATT CCC CTG GAT Phe Leu Leu Tyr Ser Val His Glu Gly Ile Arg Gly Ile Pro Leu Asp 1895 1900 1905	6187
CCC AAT GAC AAG TCA GAT GCC CTG GTC CCA GTG TCC GGG ACC TCG CTG Pro Asn Asp Lys Ser Asp Ala Leu Val Pro Val Ser Gly Thr Ser Leu 1910 1915 1920	6235
GCT GTC GGC ATC GAC TTC CAC GCT GAA AAT GAC ACC ATC TAC TGG GTG Ala Val Gly Ile Asp Phe His Ala Glu Asn Asp Thr Ile Tyr Trp Val 1925 1930 1935	6283
GAC ATG GGC CTG AGC ACG ATC AGC CGG GCC AAG CGG GAC CAG ACG TGG Asp Met Gly Leu Ser Thr Ile Ser Arg Ala Lys Arg Asp Gln Thr Trp 1940 1945 1950 1955	6331
CGT GAA GAC GTG GTG ACC AAT GGC ATT GGC CGT GTG GAG GGC ATT GCA Arg Glu Asp Val Val Thr Asn Gly Ile Gly Arg Val Glu Gly Ile Ala 1960 1965 1970	6379
GTG GAC TGG ATC GCA GGC AAC ATC TAC TGG ACA GAC CAG GGC TTT GAT Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp Thr Asp Gln Gly Phe Asp 1975 1980 1985	6427
GTC ATC GAG GTC GCC CGG CTC AAT GGC TCC TTC CGC TAC GTG GTG ATC Val Ile Glu Val Ala Arg Leu Asn Gly Ser Phe Arg Tyr Val Val Ile 1990 1995 2000	6475
TCC CAG GGT CTA GAC AAG CCC CGG GCC ATC ACC GTC CAC CCG GAG AAA Ser Gln Gly Leu Asp Lys Pro Arg Ala Ile Thr Val His Pro Glu Lys 2005 2010 2015	6523
GGG TAC TTG TTC TGG ACT GAG TGG GGT CAG TAT CCG CGT ATT GAG CGG Gly Tyr Leu Phe Trp Thr Glu Trp Gly Gln Tyr Pro Arg Ile Glu Arg 2020 2025 2030 2035	6571
TCT CGG CTA GAT GGC ACG GAG CGT GTG GTG CTG GTC AAC GTC AGC ATC Ser Arg Leu Asp Gly Thr Glu Arg Val Val Leu Val Asn Val Ser Ile 2040 2045 2050	6619
AGC TGG CCC AAC GGC ATC TCA GTG GAC TAC CAG GAT GGG AAG CTG TAC Ser Trp Pro Asn Gly Ile Ser Val Asp Tyr Gln Asp Gly Lys Leu Tyr 2055 2060 2065	6667
TGG TGC GAT GCA CGG ACA GAC AAG ATT GAA CGG ATC GAC CTG GAG ACA Trp Cys Asp Ala Arg Thr Asp Lys Ile Glu Arg Ile Asp Leu Glu Thr 2070 2075 2080	6715
GGT GAG AAC CGC GAG GTG GTT CTG TCC AGC AAC AAC ATG GAC ATG TTT Gly Glu Asn Arg Glu Val Val Leu Ser Ser Asn Asn Met Asp Met Phe 2085 2090 2095	6763
TCA GTG TCT GTG TTT GAG GAT TTC ATC TAC TGG AGT GAC AGG ACT CAT Ser Val Ser Val Phe Glu Asp Phe Ile Tyr Trp Ser Asp Arg Thr His 2100 2105 2110 2115	6811

FIG. 14A

GCC AAC GGC TCT ATC AAG CGC GGG AGC AAA GAC AAT GCC ACA GAC TCC Ala Asn Gly Ser Ile Lys Arg Gly Ser Lys Asp Asn Ala Thr Asp Ser 2120 2125 2130	6859
GTG CCC CTG CGA ACC GGC ATC GGC GTC CAG CTT AAA GAC ATC AAA GTC Val Pro Leu Arg Thr Gly Ile Gly Val Gln Leu Lys Asp Ile Lys Val 2135 2140 2145	6907
TTC AAC CGG GAC CGG CAG AAA GGC ACC AAC GTG TGC GCG GTG GCC AAT Phe Asn Arg Asp Arg Gln Lys Gly Thr Asn Val Cys Ala Val Ala Asn 2150 2155 2160	6955
GGC GGG TGC CAG CAG CTG TGC CTG TAC CGG GGC CGT GGG CAG CGG GCC Gly Gly Cys Gln Gln Leu Cys Leu Tyr Arg Gly Arg Gly Gln Arg Ala 2165 2170 2175	7003
TGC GCC TGT GCC CAC GGG ATG CTG GCT GAA GAC GGA GCA TCG TGC CGC Cys Ala Cys Ala His Gly Met Leu Ala Glu Asp Gly Ala Ser Cys Arg 2180 2185 2190 2195	7051
GAG TAT GCC GGC TAC CTG CTC TAC TCA GAG CGC ACC ATT CTC AAG AGT Glu Tyr Ala Gly Tyr Leu Leu Tyr Ser Glu Arg Thr Ile Leu Lys Ser 2200 2205 2210	7099
ATC CAC CTG TCG GAT GAG CGC AAC CTC AAT GCG CCC GTG CAG CCC TTC Ile His Leu Ser Asp Glu Arg Asn Leu Asn Ala Pro Val Gln Pro Phe 2215 2220 2225	7147
GAG GAC CCT GAG CAC ATG AAG AAC GTC ATC GCC CTG GCC TTT GAC TAC Glu Asp Pro Glu His Met Lys Asn Val Ile Ala Leu Ala Phe Asp Tyr 2230 2235 2240	7195
CGG GCA GGC ACC TCT CCG GGC ACC CCC AAT CGC ATC TTC TTC AGC GAC Arg Ala Gly Thr Ser Pro Gly Thr Pro Asn Arg Ile Phe Phe Ser Asp 2245 2250 2255	7243
ATC CAC TTT GGG AAC ATC CAA CAG ATC AAC GAC GAT GGC TCC AGG AGG Ile His Phe Gly Asn Ile Gln Gln Ile Asn Asp Asp Gly Ser Arg Arg 2260 2265 2270 2275	7291
ATC ACC ATT GTG GAA AAC GTG GGC TCC GTG GAA GGC CTG GCC TAT CAC Ile Thr Ile Val Glu Asn Val Gly Ser Val Glu Gly Leu Ala Tyr His 2280 2285 2290	7339
CGT GGC TGG GAC ACT CTC TAT TGG ACA AGC TAC ACG ACA TCC ACC ATC Arg Gly Trp Asp Thr Leu Tyr Trp Thr Ser Tyr Thr Thr Ser Thr Ile 2295 2300 2305	7387
ACG CGC CAC ACA GTG GAC CAG ACC CGC CCA GGG GCC TTC GAG CGT GAG Thr Arg His Thr Val Asp Gln Thr Arg Pro Gly Ala Phe Glu Arg Glu 2310 2315 2320	7435
ACC GTC ATC ACT ATG TCT GGA GAT GAC CAC CCA CGG GCC TTC GTT TTG Thr Val Ile Thr Met Ser Gly Asp Asp His Pro Arg Ala Phe Val Leu 2325 2330 2335	7483
GAC GAG TGC CAG AAC CTC ATG TTC TGG ACC AAC TGG AAT GAG CAG CAT Asp Glu Cys Gln Asn Leu Met Phe Trp Thr Asn Trp Asn Glu Gln His 2340 2345 2350 2355	7531

FIG. 14A

CCC AGC ATC ATG CGG GCG GCG CTC TCG GGA GCC AAT GTC CTG ACC CTT Pro Ser Ile Met Arg Ala Ala Leu Ser Gly Ala Asn Val Leu Thr Leu 2360 2365 2370	7579
ATC GAG AAG GAC ATC CGT ACC CCC AAT GGC CTG GCC ATC GAC CAC CGT Ile Glu Lys Asp Ile Arg Thr Pro Asn Gly Leu Ala Ile Asp His Arg 2375 2380 2385	7627
GCC GAG AAG CTC TAC TTC TCT GAC GCC ACC CTG GAC AAG ATC GAG CGG Ala Glu Lys Leu Tyr Phe Ser Asp Ala Thr Leu Asp Lys Ile Glu Arg 2390 2395 2400	7675
TGC GAG TAT GAC GGC TCC CAC CGC TAT GTG ATC CTA AAG TCA GAG CCT Cys Glu Tyr Asp Gly Ser His Arg Tyr Val Ile Leu Lys Ser Glu Pro 2405 2410 2415	7723
GTC CAC CCC TTC GGG CTG GCC GTG TAT GGG GAG CAC ATT TTC TGG ACT Val His Pro Phe Gly Leu Ala Val Tyr Gly Glu His Ile Phe Trp Thr 2420 2425 2430 2435	7771
GAC TGG GTG CGG CGG GCA GTG CAG CGG GCC AAC AAG CAC GTG GGC AGC Asp Trp Val Arg Arg Ala Val Gln Arg Ala Asn Lys His Val Gly Ser 2440 2445 2450	7819
AAC ATG AAG CTG CTG CGC GTG GAC ATC CCC CAG CAG CCC ATG GGC ATC Asn Met Lys Leu Leu Arg Val Asp Ile Pro Gln Gln Pro Met Gly Ile 2455 2460 2465	7867
ATC GCC GTG GCC AAC GAC ACC AAC AGC TGT GAA CTC TCT CCA TGC CGA Ile Ala Val Ala Asn Asp Thr Asn Ser Cys Glu Leu Ser Pro Cys Arg 2470 2475 2480	7915
ATC AAC AAC GGT GGC TGC CAG GAC CTG TGT CTG CTC ACT CAC CAG GGC Ile Asn Asn Gly Gly Cys Gln Asp Leu Cys Leu Leu Thr His Gln Gly 2485 2490 2495	7963
CAT GTC AAC TGC TCA TGC CGA GGG GGC CGA ATC CTC CAG GAT GAC CTC His Val Asn Cys Ser Cys Arg Gly Gly Arg Ile Leu Gln Asp Asp Leu 2500 2505 2510 2515	8011
ACC TGC CGA GCG GTG AAT TCC TCT TGC CGA GCA CAA GAT GAG TTT GAG Thr Cys Arg Ala Val Asn Ser Ser Cys Arg Ala Gln Asp Glu Phe Glu 2520 2525 2530	8059
TGT GCC AAT GGC GAG TGC ATC AAC TTC AGC CTG ACC TGC GAC GGC GTC Cys Ala Asn Gly Glu Cys Ile Asn Phe Ser Leu Thr Cys Asp Gly Val 2535 2540 2545	8107
CCC CAC TGC AAG GAC AAG TCC GAT GAG AAG CCA TCC TAC TGC AAC TCC Pro His Cys Lys Asp Lys Ser Asp Glu Lys Pro Ser Tyr Cys Asn Ser 2550 2555 2560	8155
CGC CGC TGC AAG AAG ACT TTC CGG CAG TGC AGC AAT GGG CGC TGT GTG Arg Arg Cys Lys Lys Thr Phe Arg Gln Cys Ser Asn Gly Arg Cys Val 2565 2570 2575	8203
TCC AAC ATG CTG TGG TGC AAC GGG GCC GAC GAC TGT GGG GAT GGC TCT Ser Asn Met Leu Trp Cys Asn Gly Ala Asp Asp Cys Gly Asp Gly Ser 2580 2585 2590 2595	8251

FIG. 14A

GAC Asp	GAG Glu	ATC Ile	CCT Pro	TGC Cys	AAC Asn	AAG Lys	ACA Thr	GCC Ala	TGT Cys	GGT Gly	GTG Val	GGC Gly	GAG Glu	TTC Phe	CGC Arg	8299
			2600					2605					2610			
TGC Cys	CGG Arg	GAC Asp	GGG Gly	ACC Thr	TGC Cys	ATC Ile	GGG Gly	AAC Asn	TCC Ser	AGC Ser	CGC Arg	TGC Cys	AAC Asn	CAG Gln	TTT Phe	8347
			2615					2620					2625			
GTG Val	GAT Asp	TGT Cys	GAG Glu	GAC Asp	GCC Ala	TCA Ser	GAT Asp	GAG Glu	ATG Met	AAC Asn	TGC Cys	AGT Ser	GCC Ala	ACC Thr	GAC Asp	8395
		2630					2635					2640				
TGC Cys	AGC Ser	AGC Ser	TAC Tyr	TTC Phe	CGC Arg	CTG Leu	GGC Gly	GTG Val	AAG Lys	GGC Gly	GTG Val	CTC Leu	TTC Phe	CAG Gln	CCC Pro	8443
	2645					2650					2655					
TGC Cys	GAG Glu	CGG Arg	ACC Thr	TCA Ser	CTC Leu	TGC Cys	TAC Tyr	GCA Ala	CCC Pro	AGC Ser	TGG Trp	GTG Val	TGT Cys	GAT Asp	GGC Gly	8491
2660				2665					2670						2675	
GCC Ala	AAT Asn	GAC Asp	TGT Cys	GGG Gly	GAC Asp	TAC Tyr	AGT Ser	GAT Asp	GAG Glu	CGC Arg	GAC Asp	TGC Cys	CCA Pro	GGT Gly	GTG Val	8539
			2680						2685				2690			
AAA Lys	CGC Arg	CCC Pro	AGA Arg	TGC Cys	CCT Pro	CTG Leu	AAT Asn	TAC Tyr	TTC Phe	GCC Ala	TGC Cys	CCT Pro	AGT Ser	GGG Gly	CGC Arg	8587
			2695					2700					2705			
TGC Cys	ATC Ile	CCC Pro	ATG Met	AGC Ser	TGG Trp	ACG Thr	TGT Cys	GAC Asp	AAA Lys	GAG Glu	GAT Asp	GAC Asp	TGT Cys	GAA Glu	CAT His	8635
	2710						2715					2720				
GGC Gly	GAG Glu	GAC Asp	GAG Glu	ACC Thr	CAC His	TGC Cys	AAC Asn	AAG Lys	TTC Phe	TGC Cys	TCA Ser	GAG Glu	GCC Ala	CAG Gln	TTT Phe	8683
	2725				2730						2735					
GAG Glu	TGC Cys	CAG Gln	AAC Asn	CAT His	CGC Arg	TGC Cys	ATC Ile	TCC Ser	AAG Lys	CAG Gln	TGG Trp	CTG Leu	TGT Cys	GAC Asp	GGC Gly	8731
2740				2745					2750				2755			
AGC Ser	GAT Asp	GAC Asp	TGT Cys	GGG Gly	GAT Asp	GGC Gly	TCA Ser	GAC Asp	GAG Glu	GCT Ala	GCT Ala	CAC His	TGT Cys	GAA Glu	GGC Gly	8779
			2760					2765					2770			
AAG Lys	ACG Thr	TGC Cys	GGC Gly	CCC Pro	TCC Ser	TCC Ser	TTC Phe	TCC Ser	TGC Cys	CCT Pro	GGC Gly	ACC Thr	CAC His	GTG Val	TGC Cys	8827
		2775					2780						2785			
GTC Val	CCC Pro	GAG Glu	CGC Arg	TGG Trp	CTC Leu	TGT Cys	GAC Asp	GGT Gly	GAC Asp	AAA Lys	GAC Asp	TGT Cys	GCT Ala	GAT Asp	GGT Gly	8875
	2790					2795						2800				
GCA Ala	GAC Asp	GAG Glu	AGC Ser	ATC Ile	GCA Ala	GCT Ala	GGT Gly	TGC Cys	TTG Leu	TAC Tyr	AAC Asn	AGC Ser	ACT Thr	TGT Cys	GAC Asp	8923
	2805				2810						2815					
GAC Asp	CGT Arg	GAG Glu	TTC Phe	ATG Met	TGC Cys	CAG Gln	AAC Asn	CGC Arg	CAG Gln	TGC Cys	ATC Ile	CCC Pro	AAG Lys	CAC His	TTC Phe	8971
2820				2825					2830						2835	

FIG. 14A

GTG TGT GAC CAC GAC CGT GAC TGT GCA GAT GGC TCT GAT GAG TCC CCC Val Cys Asp His Asp Arg Asp Cys Ala Asp Gly Ser Asp Glu Ser Pro 2840 2845 2850	9019
GAG TGT GAG TAC CCG ACC TGC GGC CCC AGT GAG TTC CGC TGT GCC AAT Glu Cys Glu Tyr Pro Thr Cys Gly Pro Ser Glu Phe Arg Cys Ala Asn 2855 2860 2865	9067
GGG CGC TGT CTG AGC TCC CGC CAG TGG GAG TGT GAT GGC GAG AAT GAC Gly Arg Cys Leu Ser Ser Arg Gln Trp Glu Cys Asp Gly Glu Asn Asp 2870 2875 2880	9115
TGC CAC GAC CAG AGT GAC GAG GCT CCC AAG AAC CCA CAC TGC ACC AGC Cys His Asp Gln Ser Asp Glu Ala Pro Lys Asn Pro His Cys Thr Ser 2885 2890 2895	9163
CCA GAG CAC AAG TGC AAT GCC TCG TCA CAG TTC CTG TGC AGC AGT GGG Pro Glu His Lys Cys Asn Ala Ser Ser Gln Phe Leu Cys Ser Ser Gly 2900 2905 2910 2915	9211
CGC TGT GTG GCT GAG GCA CTG CTC TGC AAC GGC CAG GAT GAC TGT GGC Arg Cys Val Ala Glu Ala Leu Leu Cys Asn Gly Gln Asp Asp Cys Gly 2920 2925 2930	9259
GAC AGC TCG GAC GAG CGT GGC TGC CAC ATC AAT GAG TGT CTC AGC CGC Asp Ser Ser Asp Glu Arg Gly Cys His Ile Asn Glu Cys Leu Ser Arg 2935 2940 2945	9307
AAG CTC AGT GGC TGC AGC CAG GAC TGT GAG GAC CTC AAG ATC GGC TTC Lys Leu Ser Gly Cys Ser Gln Asp Cys Glu Asp Leu Lys Ile Gly Phe 2950 2955 2960	9355
AAG TGC CGC TGT CGC CCT GGC TTC CGG CTG AAG GAT GAC GGC CGG ACG Lys Cys Arg Cys Arg Pro Gly Phe Arg Leu Lys Asp Asp Gly Arg Thr 2965 2970 2975	9403
TGT GCT GAT GTG GAC GAG TGC AGC ACC ACC TTC CCC TGC AGC CAG CGC Cys Ala Asp Val Asp Glu Cys Ser Thr Thr Phe Pro Cys Ser Gln Arg 2980 2985 2990 2995	9451
TGC ATC AAC ACC CAT GGC AGC TAT AAG TGT CTG TGT GTG GAG GGC TAT Cys Ile Asn Thr His Gly Ser Tyr Lys Cys Leu Cys Val Glu Gly Tyr 3000 3005 3010	9499
GCA CCC CGC GGC GGC GAC CCC CAC AGC TGC AAG GCT GTG ACT GAC GAG Ala Pro Arg Gly Gly Asp Pro His Ser Cys Lys Ala Val Thr Asp Glu 3015 3020 3025	9547
GAA CCG TTT CTG ATC TTC GCC AAC CGG TAC TAC CTG CGC AAG CTC AAC Glu Pro Phe Leu Ile Phe Ala Asn Arg Tyr Tyr Leu Arg Lys Leu Asn 3030 3035 3040	9595
CTG GAC GGG TCC AAC TAC ACG TTA CTT AAG CAG GGC CTG AAC AAC GCC Leu Asp Gly Ser Asn Tyr Thr Leu Leu Lys Gln Gly Leu Asn Asn Ala 3045 3050 3055	9643
GTT GCC TTG GAT TTT GAC TAC CGA GAG CAG ATG ATC TAC TGG ACA GAT Val Ala Leu Asp Phe Asp Tyr Arg Glu Gln Met Ile Tyr Trp Thr Asp 3060 3065 3070 3075	9691

FIG. 14A

GTG ACC ACC CAG GGC AGC ATG ATC CGA AGG ATG CAC CTT AAC GGG AGC Val Thr Thr Gln Gly Ser Met Ile Arg Arg Met His Leu Asn Gly Ser 3080 3085 3090	9739
AAT GTG CAG GTC CTA CAC CGT ACA GGC CTC AGC AAC CCC GAT GGG CTG Asn Val Gln Val Leu His Arg Thr Gly Leu Ser Asn Pro Asp Gly Leu 3095 3100 3105	9787
GCT GTG GAC TGG GTG GGT GGC AAC CTG TAC TGG TGC GAC AAA GGC CGG Ala Val Asp Trp Val Gly Gly Asn Leu Tyr Trp Cys Asp Lys Gly Arg 3110 3115 3120	9835
GAC ACC ATC GAG GTG TCC AAG CTC AAT GGG GCC TAT CGG ACG GTG CTG Asp Thr Ile Glu Val Ser Lys Leu Asn Gly Ala Tyr Arg Thr Val Leu 3125 3130 3135	9883
GTC AGC TCT GGC CTC CGT GAG CCC AGG GCT CTG GTG GTG GAT GTG CAG Val Ser Ser Gly Leu Arg Glu Pro Arg Ala Leu Val Val Asp Val Gln 3140 3145 3150 3155	9931
AAT GGG TAC CTG TAC TGG ACA GAC TGG GGT GAC CAT TCA CTG ATC GGC Asn Gly Tyr Leu Tyr Trp Thr Asp Trp Gly Asp His Ser Leu Ile Gly 3160 3165 3170	9979
CGC ATC GGC ATG GAT GGG TCC AGC CGC AGC GTC ATC GTG GAC ACC AAG Arg Ile Gly Met Asp Gly Ser Ser Arg Ser Val Ile Val Asp Thr Lys 3175 3180 3185	10027
ATC ACA TGG CCC AAT GGC CTG ACG CTG GAC TAT GTC ACT GAG CGC ATC Ile Thr Trp Pro Asn Gly Leu Thr Leu Asp Tyr Val Thr Glu Arg Ile 3190 3195 3200	10075
TAC TGG GCC GAC GCC CGC GAG GAC TAC ATT GAA TTT GCC AGC CTG GAT Tyr Trp Ala Asp Ala Arg Glu Asp Tyr Ile Glu Phe Ala Ser Leu Asp 3205 3210 3215	10123
GGC TCC AAT CGC CAC GTT GTG CTG AGC CAG GAC ATC CCG CAC ATC TTT Gly Ser Asn Arg His Val Val Leu Ser Gln Asp Ile Pro His Ile Phe 3220 3225 3230 3235	10171
GCA CTG ACC CTG TTT GAG GAC TAC GTC TAC TGG ACC GAC TGG GAA ACA Ala Leu Thr Leu Phe Glu Asp Tyr Val Tyr Trp Thr Asp Trp Glu Thr 3240 3245 3250	10219
AAG TCC ATT AAC CGA GCC CAC AAG ACC ACG GGC ACC AAC AAA ACG CTC Lys Ser Ile Asn Arg Ala His Lys Thr Thr Gly Thr Asn Lys Thr Leu 3255 3260 3265	10267
CTC ATC AGC ACG CTG CAC CGG CCC ATG GAC CTG CAT GTC TTC CAT GCC Leu Ile Ser Thr Leu His Arg Pro Met Asp Leu His Val Phe His Ala 3270 3275 3280	10315
CTG CGC CAG CCA GAC GTG CCC AAT CAC CCC TGC AAG GTC AAC AAT GGT Leu Arg Gln Pro Asp Val Pro Asn His Pro Cys Lys Val Asn Asn Gly 3285 3290 3295	10363
GGC TGC AGC AAC CTG TGC CTG CTG TCC CCC GGG GGA GGG CAC AAA TGT Gly Cys Ser Asn Leu Cys Leu Leu Ser Pro Gly Gly Gly His Lys Cys 3300 3305 3310 3315	10411

FIG. 14A

GCC TGC CCC ACC AAC TTC TAC CTG GGC AGC GAT GGG CGC ACC TGT GTG Ala Cys Pro Thr Asn Phe Tyr Leu Gly Ser Asp Gly Arg Thr Cys Val 3320 3325 3330	10459
TCC AAC TGC ACG GCT AGC CAG TTT GTA TGC AAG AAC GAC AAG TGC ATC Ser Asn Cys Thr Ala Ser Gln Phe Val Cys Lys Asn Asp Lys Cys Ile 3335 3340 3345	10507
CCC TTC TGG TGG AAG TGT GAC ACC GAG GAC GAC TGC GGG GAC CAC TCA Pro Phe Trp Trp Lys Cys Asp Thr Glu Asp Asp Cys Gly Asp His Ser 3350 3355 3360	10555
GAC GAG CCC CCG GAC TGC CCT GAG TTC AAG TGC CGG CCC GGA CAG TTC Asp Glu Pro Pro Asp Cys Pro Glu Phe Lys Cys Arg Pro Gly Gln Phe 3365 3370 3375	10603
CAG TGC TCC ACA GGT ATC TGC ACA AAC CCT GCC TTC ATC TGC GAT GGC Gln Cys Ser Thr Gly Ile Cys Thr Asn Pro Ala Phe Ile Cys Asp Gly 3380 3385 3390 3395	10651
GAC AAT GAC TGC CAG GAC AAC AGT GAC GAG GCC AAC TGT GAC ATC CAC Asp Asn Asp Cys Gln Asp Asn Ser Asp Glu Ala Asn Cys Asp Ile His 3400 3405 3410	10699
GTC TGC TTG CCC AGT CAG TTC AAA TGC ACC AAC ACC AAC CGC TGT ATT Val Cys Leu Pro Ser Gln Phe Lys Cys Thr Asn Thr Asn Arg Cys Ile 3415 3420 3425	10747
CCC GGC ATC TTC CGC TGC AAT GGG CAG GAC AAC TGC GGA GAT GGG GAG Pro Gly Ile Phe Arg Cys Asn Gly Gln Asp Asn Cys Gly Asp Gly Glu 3430 3435 3440	10795
GAT GAG AGG GAC TGC CCC GAG GTG ACC TGC GCC CCC AAC CAG TTC CAG Asp Glu Arg Asp Cys Pro Glu Val Thr Cys Ala Pro Asn Gln Phe Gln 3445 3450 3455	10843
TGC TCC ATT ACC AAA CGG TGC ATC CCC CGG GTC TGG GTC TGC GAC CGG Cys Ser Ile Thr Lys Arg Cys Ile Pro Arg Val Trp Val Cys Asp Arg 3460 3465 3470 3475	10891
GAC AAT GAC TGT GTG GAT GGC AGT GAT GAG CCC GCC AAC TGC ACC CAG Asp Asn Asp Cys Val Asp Gly Ser Asp Glu Pro Ala Asn Cys Thr Gln 3480 3485 3490	10939
ATG ACC TGT GGT GTG GAC GAG TTC CGC TGC AAG GAT TCG GGC CGC TGC Met Thr Cys Gly Val Asp Glu Phe Arg Cys Lys Asp Ser Gly Arg Cys 3495 3500 3505	10987
ATC CCA GCG CGT TGG AAG TGT GAC GGA GAG GAT GAC TGT GGG GAT GGC Ile Pro Ala Arg Trp Lys Cys Asp Gly Glu Asp Asp Cys Gly Asp Gly 3510 3515 3520	11035
TCG GAT GAG CCC AAG GAA GAG TGT GAT GAA CGC ACC TGT GAG CCA TAC Ser Asp Glu Pro Lys Glu Glu Cys Asp Glu Arg Thr Cys Glu Pro Tyr 3525 3530 3535	11083
CAG TTC CGC TGC AAG AAC AAC CGC TGC GTG CCC GGC CGC TGG CAG TGC Gln Phe Arg Cys Lys Asn Asn Arg Cys Val Pro Gly Arg Trp Gln Cys 3540 3545 3550 3555	11131

FIG. 14A

GAC TAC GAC AAC GAT TGC GGT GAC AAC TCC GAT GAA GAG AGC TGC ACC Asp Tyr Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Glu Ser Cys Thr 3560 3565 3570	11179
CCT CGG CCC TGC TCC GAG AGT GAG TTC TCC TGT GCC AAC GGC CGC TGC Pro Arg Pro Cys Ser Glu Ser Glu Phe Ser Cys Ala Asn Gly Arg Cys 3575 3580 3585	11227
ATC GCG GGG CGC TGG AAA TGC GAT GGA GAC CAC GAC TGC GCG GAC GGC Ile Ala Gly Arg Trp Lys Cys Asp Gly Asp His Asp Cys Ala Asp Gly 3590 3595 3600	11275
TCG GAC GAG AAA GAC TGC ACC CCC CGC TGT GAC ATG GAC CAG TTC CAG Ser Asp Glu Lys Asp Cys Thr Pro Arg Cys Asp Met Asp Gln Phe Gln 3605 3610 3615	11323
TGC AAG AGC GGC CAC TGC ATC CCC CTG CGC TGG CGC TGT GAC GCA GAC Cys Lys Ser Gly His Cys Ile Pro Leu Arg Trp Arg Cys Asp Ala Asp 3620 3625 3630 3635	11371
GCC GAC TGC ATG GAC GGC AGC GAC GAG GAG GCC TGC GGC ACT GGC GTG Ala Asp Cys Met Asp Gly Ser Asp Glu Glu Ala Cys Gly Thr Gly Val 3640 3645 3650	11419
CGG ACC TGC CCC CTG GAC GAG TTC CAG TGC AAC AAC ACC TTG TGC AAG Arg Thr Cys Pro Leu Asp Glu Phe Gln Cys Asn Asn Thr Leu Cys Lys 3655 3660 3665	11467
CCG CTG GCC TGG AAG TGC GAT GGC GAG GAT GAC TGT GGG GAC AAC TCA Pro Leu Ala Trp Lys Cys Asp Gly Glu Asp Asp Cys Gly Asp Asn Ser 3670 3675 3680	11515
GAT GAG AAC CCC GAG GAG TGT GCC CGG TTC GTG TGC CCT CCC AAC CGG Asp Glu Asn Pro Glu Glu Cys Ala Arg Phe Val Cys Pro Pro Asn Arg 3685 3690 3695	11563
CCC TTC CGT TGC AAG AAT GAC CGC GTC TGT CTG TGG ATC GGG CGC CAA Pro Phe Arg Cys Lys Asn Asp Arg Val Cys Leu Trp Ile Gly Arg Gln 3700 3705 3710 3715	11611
TGC GAT GGC ACG GAC AAC TGT GGG GAT GGG ACT GAT GAA GAG GAC TGT Cys Asp Gly Thr Asp Asn Cys Gly Asp Gly Thr Asp Glu Glu Asp Cys 3720 3725 3730	11659
GAG CCC CCC ACA GCC CAC ACC ACC CAC TGC AAA GAC AAG AAG GAG TTT Glu Pro Pro Thr Ala His Thr Thr His Cys Lys Asp Lys Lys Glu Phe 3735 3740 3745	11707
CTG TGC CGG AAC CAG CGC TGC CTC TCC TCC TCC CTG CGC TGC AAC ATG Leu Cys Arg Asn Gln Arg Cys Leu Ser Ser Ser Leu Arg Cys Asn Met 3750 3755 3760	11755
TTC GAT GAC TGC GGG GAC GGC TCT GAC GAG GAG GAC TGC AGC ATC GAC Phe Asp Asp Cys Gly Asp Gly Ser Asp Glu Glu Asp Cys Ser Ile Asp 3765 3770 3775	11803
CCC AAG CTG ACC AGC TGC GCC ACC AAT GCC AGC ATC TGT GGG GAC GAG Pro Lys Leu Thr Ser Cys Ala Thr Asn Ala Ser Ile Cys Gly Asp Glu 3780 3785 3790 3795	11851

FIG. 14A

GCA CGC TGC GTG CGC ACC GAG AAA GCG GCC TAC TGT GCC TGC CGC TCG Ala Arg Cys Val Arg Thr Glu Lys Ala Ala Tyr Cys Ala Cys Arg Ser 3800 3805 3810	11899
GGC TTC CAC ACC GTG CCC GGC CAG CCC GGA TGC CAA GAC ATC AAC GAG Gly Phe His Thr Val Pro Gly Gln Pro Gly Cys Gln Asp Ile Asn Glu 3815 3820 3825	11947
TGC CTG CGC TTC GGC ACC TGC TCC CAG CTC TGC AAC AAC ACC AAG GGC Cys Leu Arg Phe Gly Thr Cys Ser Gln Leu Cys Asn Asn Thr Lys Gly 3830 3835 3840	11995
GGC CAC CTC TGC AGC TGC GCT CGG AAC TTC ATG AAG ACG CAC AAC ACC Gly His Leu Cys Ser Cys Ala Arg Asn Phe Met Lys Thr His Asn Thr 3845 3850 3855	12043
TGC AAG GCC GAA GGC TCT GAG TAC CAG GTC CTG TAC ATC GCT GAT GAC Cys Lys Ala Glu Gly Ser Glu Tyr Gln Val Leu Tyr Ile Ala Asp Asp 3860 3865 3870 3875	12091
AAT GAG ATC CGC AGC CTG TTC CCC GGC CAC CCC CAT TCG GCT TAC GAG Asn Glu Ile Arg Ser Leu Phe Pro Gly His Pro His Ser Ala Tyr Glu 3880 3885 3890	12139
CAG GCA TTC CAG GGT GAC GAG AGT GTC CGC ATT GAT GCT ATG GAT GTC Gln Ala Phe Gln Gly Asp Glu Ser Val Arg Ile Asp Ala Met Asp Val 3895 3900 3905	12187
CAT GTC AAG GCT GGC CGT GTC TAT TGG ACC AAC TGG CAC ACG GGC ACC His Val Lys Ala Gly Arg Val Tyr Trp Thr Asn Trp His Thr Gly Thr 3910 3915 3920	12235
ATC TCC TAC CGC AGC CTG CCA CCT GCT GCG CCT CCT ACC ACT TCC AAC Ile Ser Tyr Arg Ser Leu Pro Pro Ala Ala Pro Pro Thr Thr Ser Asn 3925 3930 3935	12283
CGC CAC CGG CGA CAG ATT GAC CGG GGT GTC ACC CAC CTC AAC ATT TCA Arg His Arg Arg Gln Ile Asp Arg Gly Val Thr His Leu Asn Ile Ser 3940 3945 3950 3955	12331
GGG CTG AAG ATG CCC AGA GGC ATC GCC ATC GAC TGG GTG GCC GGA AAC Gly Leu Lys Met Pro Arg Gly Ile Ala Ile Asp Trp Val Ala Gly Asn 3960 3965 3970	12379
GTG TAC TGG ACC GAC TCG GGC CGA GAT GTG ATT GAG GTG GCG CAG ATG Val Tyr Trp Thr Asp Ser Gly Arg Asp Val Ile Glu Val Ala Gln Met 3975 3980 3985	12427
AAG GGC GAG AAC CGC AAG ACG CTC ATC TCG GGC ATG ATT GAC GAG CCC Lys Gly Glu Asn Arg Lys Thr Leu Ile Ser Gly Met Ile Asp Glu Pro 3990 3995 4000	12475
CAC GCC ATT GTG GTG GAC CCA CTG AGG GGG ACC ATG TAC TGG TCA GAC His Ala Ile Val Val Asp Pro Leu Arg Gly Thr Met Tyr Trp Ser Asp 4005 4010 4015	12523
TGG GGC AAC CAC CCC AAG ATT GAG ACG GCA GCG ATG GAT GGG ACG CTT Trp Gly Asn His Pro Lys Ile Glu Thr Ala Ala Met Asp Gly Thr Leu 4020 4025 4030 4035	12571

FIG. 14A

CGG GAG ACA CTG GTG CAG GAC AAC ATT CAG TGG CCC ACA GGC CTG GCC	12619
Arg Glu Thr Leu Val Gln Asp Asn Ile Gln Trp Pro Thr Gly Leu Ala	
4040 4045 4050	
GTG GAT TAT CAC AAT GAG CGG CTG TAC TGG GCA GAC GCC AAG CTT TCA	12667
Val Asp Tyr His Asn Glu Arg Leu Tyr Trp Ala Asp Ala Lys Leu Ser	
4055 4060 4065	
GTC ATC GGC AGC ATC CGG CTC AAT GGC ACG GAC CCC ATT GTG GCT GCT	12715
Val Ile Gly Ser Ile Arg Leu Asn Gly Thr Asp Pro Ile Val Ala Ala	
4070 4075 4080	
GAC AGC AAA CGA GGC CTA AGT CAC CCC TTC AGC ATC GAC GTC TTT GAG	12763
Asp Ser Lys Arg Gly Leu Ser His Pro Phe Ser Ile Asp Val Phe Glu	
4085 4090 4095	
GAT TAC ATC TAT GGT GTC ACC TAC ATC AAT AAT CGT GTC TTC AAG ATC	12811
Asp Tyr Ile Tyr Gly Val Thr Tyr Ile Asn Asn Arg Val Phe Lys Ile	
4100 4105 4110 4115	
CAT AAG TTT GGC CAC AGC CCC TTG GTC AAC CTG ACA GGG GGC CTG AGC	12859
His Lys Phe Gly His Ser Pro Leu Val Asn Leu Thr Gly Gly Leu Ser	
4120 4125 4130	
CAC GCC TCT GAC GTG GTC CTT TAC CAT CAG CAC AAG CAG CCC GAA GTG	12907
His Ala Ser Asp Val Val Leu Tyr His Gln His Lys Gln Pro Glu Val	
4135 4140 4145	
ACC AAC CCA TGT GAC CGC AAG AAA TGC GAG TGG CTC TGC CTG CTG AGC	12955
Thr Asn Pro Cys Asp Arg Lys Lys Cys Glu Trp Leu Cys Leu Leu Ser	
4150 4155 4160	
CCC AGT GGG CCT GTC TGC ACC TGT CCC AAT GGG AAG CGG CTG GAC AAC	13003
Pro Ser Gly Pro Val Cys Thr Cys Pro Asn Gly Lys Arg Leu Asp Asn	
4165 4170 4175	
GGC ACA TGC GTG CCT GTG CCC TCT CCA ACG CCC CCC CCA GAT GCT CCC	13051
Gly Thr Cys Val Pro Val Pro Ser Pro Thr Pro Pro Pro Asp Ala Pro	
4180 4185 4190 4195	
CGG CCT GGA ACC TGT AAC CTG CAG TGC TTC AAC GGT GGC AGC TGT TTC	13099
Arg Pro Gly Thr Cys Asn Leu Gln Cys Phe Asn Gly Gly Ser Cys Phe	
4200 4205 4210	
CTC AAT GCA CGG AGG CAG CCC AAG TGC CGC TGC CAA CCC CGC TAC ACG	13147
Leu Asn Ala Arg Arg Gln Pro Lys Cys Arg Cys Gln Pro Arg Tyr Thr	
4215 4220 4225	
GGT GAC AAG TGT GAA CTG GAC CAG TGC TGG GAG CAC TGT CGC AAT GGG	13195
Gly Asp Lys Cys Glu Leu Asp Gln Cys Trp Glu His Cys Arg Asn Gly	
4230 4235 4240	
GGC ACC TGT GCT GCC TCC CCC TCT GGC ATG CCC ACG TGC CGG TGC CCC	13243
Gly Thr Cys Ala Ala Ser Pro Ser Gly Met Pro Thr Cys Arg Cys Pro	
4245 4250 4255	
ACG GGC TTC ACG GGC CCC AAA TGC ACC CAG CAG GTG TGT GCG GGC TAC	13291
Thr Gly Phe Thr Gly Pro Lys Cys Thr Gln Gln Val Cys Ala Gly Tyr	
4260 4265 4270 4275	

FIG. 14A

TGT Cys	GCC Ala	AAC Asn	AAC Asn	AGC Ser	ACC Thr	TGC Cys	ACT Thr	GTC Val	AAC Asn	CAG Gln	GGC Gly	AAC Asn	CAG Gln	CCC Pro	CAG Gln	13339
			4280					4285						4290		
TGC Cys	CGA Arg	TGC Cys	CTA Leu	CCC Pro	GGC Gly	TTC Phe	CTG Leu	GGC Gly	GAC Asp	CGC Arg	TGC Cys	CAG Gln	TAC Tyr	CGG Arg	CAG Gln	13387
			4295					4300					4305			
TGC Cys	TCT Ser	GGC Gly	TAC Tyr	TGT Cys	GAG Glu	AAC Asn	TTT Phe	GGC Gly	ACA Thr	TGC Cys	CAG Gln	ATG Met	GCT Ala	GCT Ala	GAT Asp	13435
		4310					4315					4320				
GGC Gly	TCC Ser	CGA Arg	CAA Gln	TGC Cys	CGC Arg	TGC Cys	ACT Thr	GCC Ala	TAC Tyr	TTT Phe	GAG Glu	GGA Gly	TCG Ser	AGG Arg	TGT Cys	13483
							4330				4335					
GAG Glu	GTG Val	AAC Asn	AAG Lys	TGC Cys	AGC Ser	CGC Arg	TGT Cys	CTC Leu	GAA Glu	GGG Ala	GCC Cys	TGT Val	GTG Val	GTC Val	AAC Asn	13531
4340						4345				4350					4355	
AAG Lys	CAG Gln	AGT Ser	GGG Gly	GAT Asp	GTC Val	ACC Thr	TGC Cys	AAC Asn	TGC Cys	ACG Thr	GAT Asp	GGC Gly	CGG Arg	GTG Val	GCC Ala	13579
				4360					4365					4370		
CCC Pro	AGC Ser	TGT Cys	CTG Leu	ACC Thr	TGC Cys	GTC Val	GGC Gly	CAC His	TGC Cys	AGC Ser	AAT Asn	GGC Gly	GGC Gly	TCC Ser	TGT Cys	13627
			4375					4380					4385			
ACC Thr	ATG Met	AAC Asn	AGC Ser	AAA Lys	ATG Met	ATG Met	CCT Pro	GAG Glu	TGC Cys	CAG Gln	TGC Cys	CCA Pro	CCC Pro	CAC His	ATG Met	13675
		4390					4395					4400				
ACA Thr	GGG Gly	CCC Pro	CGG Arg	TGT Cys	GAG Glu	GAG Glu	CAC His	GTC Val	TTC Phe	AGC Ser	CAG Gln	CAG Gln	CAG Gln	CCA Pro	GGA Gly	13723
		4405					4410				4415					
CAT His	ATA Ile	GCC Ala	TCC Ser	ATC Ile	CTA Leu	ATC Ile	CCT Pro	CTG Leu	CTG Leu	TTG Leu	CTG Leu	CTG Leu	CTG Leu	CTG Leu	GTT Val	13771
4420					4425					4430					4435	
CTG Leu	GTG Val	GCC Ala	GGA Gly	GTG Val	GTA Val	TTC Phe	TGG Trp	TAT Tyr	AAG Lys	CGG Arg	CGA Arg	GTC Val	CAA Gln	GGG Gly	GCT Ala	13819
			4440						4445					4450		
AAG Lys	GGC Gly	TTC Phe	CAG Gln	CAC His	CAA Gln	CGG Arg	ATG Met	ACC Thr	AAC Asn	GGG Gly	GCC Ala	ATG Met	AAC Asn	GTG Val	GAG Glu	13867
			4455					4460					4465			
ATT Ile	GGA Gly	AAC Asn	CCC Pro	ACC Thr	TAC Tyr	AAG Lys	ATG Met	TAC Tyr	GAA Glu	GGC Gly	GGA Gly	GAG Glu	CCT Pro	GAT Asp	GAT Asp	13915
		4470					4475					4480				
GTG Val	GGA Gly	GGC Gly	CTA Leu	CTG Leu	GAC Asp	GCT Ala	GAC Asp	TTT Phe	GCC Ala	CTG Leu	GAC Asp	CCT Pro	GAC Asp	AAG Lys	CCC Pro	13963
		4485				4490				4495						
ACC Thr	AAC Asn	TTC Phe	ACC Thr	AAC Asn	CCC Pro	GTG Val	TAT Tyr	GCC Ala	ACA Thr	CTC Leu	TAC Tyr	ATG Met	GGG Gly	GGC Gly	CAT His	14011
4500					4505					4510					4515	

FIG. 14A

GGC AGT CGC CAC TCC CTG GCC AGC ACG GAC GAG AAG CGA GAA CTC CTG	14059
Gly Ser Arg His Ser Leu Ala Ser Thr Asp Glu Lys Arg Glu Leu Leu	
4520 4525 4530	
GGC CGG GGC CCT GAG GAC GAG ATA GGG GAC CCC TTG GCA TAGGGCCCTG CC	14110
CCGTCGGACT GCCCCAGAA AGCCTCCTGC CCCCTGCCGG TGAAGTCCTT CAGTGAGCCC	14170
Gly Arg Gly Pro Glu Asp Glu Ile Gly Asp Pro Leu Ala	
4535 4540	
CTCCCCAGCC AGCCCTTCCC TGGCCCCGCC GGATGTATAA ATGTAAAAAT GAAGGAATTA	14230
CATTTTATAT GTGAGCGAGC AAGCCGGCAA GCGAGCACAG TATTATTCT CCATCCCCTC	14290
CCTGCCTGCT CCTTGGCACC CCCATGCTGC CTTGAGGGAG ACAGGCAGGG AGGGCTTGGG	14350
GCTGCACCTC CTACCCTCCC ACCAGAACGC ACCCCACTGG GAGAGCTGGT GGTGCAGCCT	14410
TCCCCTCCCT GTATAAGACA CTTTGCCAAG GCTCTCCCCT CTCGCCCCAT CCCTGCTTGC	14470
CCGCTCCCAC AGCTTCCTGA GGGCTAATC TGGGAAGGGA GAGTCTTTG CTGCCCTGT	14530
CTGGAAGACG TGGCTCTGGG TGAGGTAGG GGGAAAGGAT GGAGTGTTT AGTTCTTGGG	14590
GGAGGCCACC CCAAACCCCA GCCCAACTC CAGGGGCACC TATGAGATGG CCATGCTCAA	14650
CCCCCTCCC AGACAGGCC TCCCTGTCTC CAGGGCCCC ACCGAGGTC CCAGGGCTGG	14710
AGACTTCCTC TGGTAAACAT TCCTCCAGCC TCCCCTCCCC TGGGGACGCC AAGGAGGTGG	14770
GCCACACCCA GGAAGGGAAA GCGGGCAGCC CCGTTTGGG GACGTGAACG TTTAATAAT	14830
TTTGCTGAA TTCTTTACAA CTAAATAACA CAGATATTCT TATAAATAAA ATTGTAAAA	14890
AAAAAA	14896

FIG. 14A

Met	Leu	Thr	Pro	Pro	Leu	Leu	Leu	Leu	Leu	Pro	Leu	Leu	Ser	Ala	Leu
1				5					10					15	
Val	Ala	Ala	Ala	Ile	Asp	Ala	Pro	Lys	Thr	Cys	Ser	Pro	Lys	Gln	Phe
			20					25					30		
Ala	Cys	Arg	Asp	Gln	Ile	Thr	Cys	Ile	Ser	Lys	Gly	Trp	Arg	Cys	Asp
		35					40					45			
Gly	Glu	Arg	Asp	Cys	Pro	Asp	Gly	Ser	Asp	Glu	Ala	Pro	Glu	Ile	Cys
		50				55						60			
Pro	Gln	Ser	Lys	Ala	Gln	Arg	Cys	Gln	Pro	Asn	Glu	His	Asn	Cys	Leu
65					70					75					80
Gly	Thr	Glu	Leu	Cys	Val	Pro	Met	Ser	Arg	Leu	Cys	Asn	Gly	Val	Gln
				85					90						95
Asp	Cys	Met	Asp	Gly	Ser	Asp	Glu	Gly	Pro	His	Cys	Arg	Glu	Leu	Gln
			100					105					110		
Gly	Asn	Cys	Ser	Arg	Leu	Gly	Cys	Gln	His	His	Cys	Val	Pro	Thr	Leu
		115						120				125			
Asp	Gly	Pro	Thr	Cys	Tyr	Cys	Asn	Ser	Ser	Phe	Gln	Leu	Gln	Ala	Asp
	130					135					140				
Gly	Lys	Thr	Cys	Lys	Asp	Phe	Asp	Glu	Cys	Ser	Val	Tyr	Gly	Thr	Cys
145					150						155				160
Ser	Gln	Leu	Cys	Thr	Asn	Thr	Asp	Gly	Ser	Phe	Ile	Cys	Gly	Cys	Val
				165				170					175		
Glu	Gly	Tyr	Leu	Leu	Gln	Pro	Asp	Asn	Arg	Ser	Cys	Lys	Ala	Lys	Asn
		180						185					190		
Glu	Pro	Val	Asp	Arg	Pro	Pro	Val	Leu	Leu	Ile	Ala	Asn	Ser	Gln	Asn
		195					200					205			
Ile	Leu	Ala	Thr	Tyr	Leu	Ser	Gly	Ala	Gln	Val	Ser	Thr	Ile	Thr	Pro
	210					215						220			
Thr	Ser	Thr	Arg	Gln	Thr	Thr	Ala	Met	Asp	Phe	Ser	Tyr	Ala	Asn	Glu
225					230					235					240
Thr	Val	Cys	Trp	Val	His	Val	Gly	Asp	Ser	Ala	Ala	Gln	Thr	Gln	Leu
				245					250				255		
Lys	Cys	Ala	Arg	Met	Pro	Gly	Leu	Lys	Gly	Phe	Val	Asp	Glu	His	Thr
			260					265					270		
Ile	Asn	Ile	Ser	Leu	Ser	Leu	His	His	Val	Glu	Gln	Met	Ala	Ile	Asp
	275						280					285			
Trp	Leu	Thr	Gly	Asn	Phe	Tyr	Phe	Val	Asp	Asp	Ile	Asp	Asp	Arg	Ile
	290					295					300				
Phe	Val	Cys	Asn	Arg	Asn	Gly	Asp	Thr	Cys	Val	Thr	Leu	Leu	Asp	Leu
305					310					315					320
Glu	Leu	Tyr	Asn	Pro	Lys	Gly	Ile	Ala	Leu	Asp	Pro	Ala	Met	Gly	Lys
				325						330				335	
Val	Phe	Phe	Thr	Asp	Tyr	Gly	Gln	Ile	Pro	Lys	Val	Glu	Arg	Cys	Asp
			340					345					350		
Met	Asp	Gly	Gln	Asn	Arg	Thr	Lys	Leu	Val	Asp	Ser	Lys	Ile	Val	Phe
		355					360					365			
Pro	His	Gly	Ile	Thr	Leu	Asp	Leu	Val	Ser	Arg	Leu	Val	Tyr	Trp	Ala
	370					375					380				
Asp	Ala	Tyr	Leu	Asp	Tyr	Ile	Glu	Val	Val	Asp	Tyr	Glu	Gly	Lys	Gly
385					390					395					400
Arg	Gln	Thr	Ile	Ile	Gln	Gly	Ile	Leu	Ile	Glu	His	Leu	Tyr	Gly	Leu
				405						410				415	
Thr	Val	Phe	Glu	Asn	Tyr	Leu	Tyr	Ala	Thr	Asn	Ser	Asp	Asn	Ala	Asn
			420					425					430		
Ala	Gln	Gln	Lys	Thr	Ser	Val	Ile	Arg	Val	Asn	Arg	Phe	Asn	Ser	Thr
			435					440				445			
Glu	Tyr	Gln	Val	Val	Thr	Arg	Val	Asp	Lys	Gly	Gly	Ala	Leu	His	Ile
	450					455					460				

FIG. 14B

Tyr His Gln Arg Arg Gln Pro Arg Val Arg Ser His Ala Cys Glu Asn  
 465 470 475 480  
 Asp Gln Tyr Gly Lys Pro Gly Gly Cys Ser Asp Ile Cys Leu Leu Ala  
 485 490 495  
 Asn Ser His Lys Ala Arg Thr Cys Arg Cys Arg Ser Gly Phe Ser Leu  
 500 505 510  
 Gly Ser Asp Gly Lys Ser Cys Lys Lys Pro Glu His Glu Leu Phe Leu  
 515 520 525  
 Val Tyr Gly Lys Gly Arg Pro Gly Ile Ile Arg Gly Met Asp Met Gly  
 530 535 540  
 Ala Lys Val Pro Asp Glu His Met Ile Pro Ile Glu Asn Leu Met Asn  
 545 550 555 560  
 Pro Arg Ala Leu Asp Phe His Ala Glu Thr Gly Phe Ile Tyr Phe Ala  
 565 570 575  
 Asp Thr Thr Ser Tyr Leu Ile Gly Arg Gln Lys Ile Asp Gly Thr Glu  
 580 585 590  
 Arg Glu Thr Ile Leu Lys Asp Gly Ile His Asn Val Glu Gly Val Ala  
 595 600 605  
 Val Asp Trp Met Gly Asp Asn Leu Tyr Trp Thr Asp Asp Gly Pro Lys  
 610 615 620  
 Lys Thr Ile Ser Val Ala Arg Leu Glu Lys Ala Ala Gln Thr Arg Lys  
 625 630 635 640  
 Thr Leu Ile Glu Gly Lys Met Thr His Pro Arg Ala Ile Val Val Asp  
 645 650 655  
 Pro Leu Asn Gly Trp Met Tyr Trp Thr Asp Trp Glu Glu Asp Pro Lys  
 660 665 670  
 Asp Ser Arg Arg Gly Arg Leu Glu Arg Ala Trp Met Asp Gly Ser His  
 675 680 685  
 Arg Asp Ile Phe Val Thr Ser Lys Thr Val Leu Trp Pro Asn Gly Leu  
 690 695 700  
 Ser Leu Asp Ile Pro Ala Gly Arg Leu Tyr Trp Val Asp Ala Phe Tyr  
 705 710 715 720  
 Asp Arg Ile Glu Thr Ile Leu Leu Asn Gly Thr Asp Arg Lys Ile Val  
 725 730 735  
 Tyr Glu Gly Pro Glu Leu Asn His Ala Phe Gly Leu Cys His His Gly  
 740 745 750  
 Asn Tyr Leu Phe Trp Thr Glu Tyr Arg Ser Gly Ser Val Tyr Arg Leu  
 755 760 765  
 Glu Arg Gly Val Gly Gly Ala Pro Pro Thr Val Thr Leu Leu Arg Ser  
 770 775 780  
 Glu Arg Pro Pro Ile Phe Glu Ile Arg Met Tyr Asp Ala Gln Gln Gln  
 785 790 795 800  
 Gln Val Gly Thr Asn Lys Cys Arg Val Asn Asn Gly Gly Cys Ser Ser  
 805 810 815  
 Leu Cys Leu Ala Thr Pro Gly Ser Arg Gln Cys Ala Cys Ala Glu Asp  
 820 825 830  
 Gln Val Leu Asp Ala Asp Gly Val Thr Cys Leu Ala Asn Pro Ser Tyr  
 835 840 845  
 Val Pro Pro Pro Gln Cys Gln Pro Gly Glu Phe Ala Cys Ala Asn Ser  
 850 855 860  
 Arg Cys Ile Gln Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys Leu  
 865 870 875 880  
 Asp Asn Ser Asp Glu Ala Pro Ala Leu Cys His Gln His Thr Cys Pro  
 885 890 895  
 Ser Asp Arg Phe Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg Trp  
 900 905 910  
 Leu Cys Asp Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser Asn  
 915 920 925

FIG. 14B

Ala Thr Cys Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys Ala  
 930 935 940  
Ser Gly Arg Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp Asp  
 945 950 955 960  
Cys Gly Asp Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro Thr Cys  
 965 970 975  
Phe Pro Leu Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile Asn Ile  
 980 985 990  
Asn Trp Arg Cys Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu  
 995 1000 1005  
Ala Gly Cys Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys Asn Ser  
 1010 1015 1020  
Gly Arg Cys Ile Pro Glu His Trp Thr Cys Asp Gly Asp Asn Asp Cys  
 025 1030 1035 1040  
Gly Asp Tyr Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln Ala Thr  
 1045 1050 1055  
Arg Pro Pro Gly Gly Cys His Thr Asp Glu Phe Gln Cys Arg Leu Asp  
 1060 1065 1070  
Gly Leu Cys Ile Pro Leu Arg Trp Arg Cys Asp Gly Asp Thr Asp Cys  
 1075 1080 1085  
Met Asp Ser Ser Asp Glu Lys Ser Cys Glu Gly Val Thr His Val Cys  
 1090 1095 1100  
Asp Pro Ser Val Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys Ile Ser  
 105 1110 1115 1120  
Lys Ala Trp Val Cys Asp Gly Asp Asn Asp Cys Glu Asp Asn Ser Asp  
 1125 1130 1135  
Glu Glu Asn Cys Glu Ser Leu Ala Cys Arg Pro Pro Ser His Pro Cys  
 1140 1145 1150  
Ala Asn Asn Thr Ser Val Cys Leu Pro Pro Asp Lys Leu Cys Asp Gly  
 1155 1160 1165  
Asn Asp Asp Cys Glv Asp Glv Ser Asp Glu Gly Glu Leu Cys Asp Gln  
 1170 1175 1180  
Cys Ser Leu Asn Asn Gly Gly Cys Ser His Asn Cys Ser Val Ala Pro  
 185 1190 1195 1200  
Gly Glu Gly Ile Val Cys Ser Cys Pro Leu Gly Met Glu Leu Gly Pro  
 1205 1210 1215  
Asp Asn His Thr Cys Gln Ile Gln Ser Tyr Cys Ala Lys His Leu Lys  
 1220 1225 1230  
Cys Ser Gln Lys Cys Asp Gln Asn Lys Phe Ser Val Lys Cys Ser Cys  
 1235 1240 1245  
Tyr Glu Gly Trp Val Leu Glu Pro Asp Gly Glu Ser Cys Arg Ser Leu  
 1250 1255 1260  
Asp Pro Phe Lys Pro Phe Ile Ile Phe Ser Asn Arg His Glu Ile Arg  
 265 1270 1275 1280  
Arg Ile Asp Leu His Lys Gly Asp Tyr Ser Val Leu Val Pro Gly Leu  
 1285 1290 1295  
Arg Asn Thr Ile Ala Leu Asp Phe His Leu Ser Gln Ser Ala Leu Tyr  
 1300 1305 1310  
Trp Thr Asp Val Val Glu Asp Lys Ile Tyr Arg Gly Lys Leu Leu Asp  
 1315 1320 1325  
Asn Gly Ala Leu Thr Ser Phe Glu Val Val Ile Gln Tyr Gly Leu Ala  
 1330 1335 1340  
Thr Pro Glu Gly Leu Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp  
 345 1350 1355 1360  
Val Glu Ser Asn Leu Asp Gln Ile Glu Val Ala Lys Leu Asp Gly Thr  
 1365 1370 1375  
Leu Arg Thr Thr Leu Leu Ala Gly Asp Ile Glu His Pro Arg Ala Ile  
 1380 1385 1390

FIG. 14B

Ala Leu Asp Pro Arg Asp Gly Ile Leu Phe Trp Thr Asp Trp Asp Ala  
 1395 1400 1405  
 Ser Leu Pro Arg Ile Glu Ala Ala Ser Met Ser Gly Ala Gly Arg Arg  
 1410 1415 1420  
 Thr Val His Arg Glu Thr Gly Ser Gly Gly Trp Pro Asn Gly Leu Thr  
 425 1430 1435 1440  
 Val Asp Tyr Leu Glu Lys Arg Ile Leu Trp Ile Asp Ala Arg Ser Asp  
 1445 1450 1455  
 Ala Ile Tyr Ser Ala Arg Tyr Asp Gly Ser Gly His Met Glu Val Leu  
 1460 1465 1470  
 Arg Gly His Glu Phe Leu Ser His Pro Phe Ala Val Thr Leu Tyr Gly  
 1475 1480 1485  
 Gly Glu Val Tyr Trp Thr Asp Trp Arg Thr Asn Thr Leu Ala Lys Ala  
 1490 1495 1500  
 Asn Lys Trp Thr Gly His Asn Val Thr Val Val Gln Arg Thr Asn Thr  
 505 1510 1515 1520  
 Gln Pro Phe Asp Leu Gln Val Tyr His Pro Ser Arg Gln Pro Met Ala  
 1525 1530 1535  
 Pro Asn Pro Cys Glu Ala Asn Gly Gly Gln Gly Pro Cys Ser His Leu  
 1540 1545 1550  
 Cys Leu Ile Asn Tyr Asn Arg Thr Val Ser Cys Ala Cys Pro His Leu  
 1555 1560 1565  
 Met Lys Leu His Lys Asp Asn Thr Thr Cys Tyr Glu Phe Lys Lys Phe  
 1570 1575 1580  
 Leu Leu Tyr Ala Arg Gln Met Glu Ile Arg Gly Val Asp Leu Asp Ala  
 585 1590 1595 1600  
 Pro Tyr Tyr Asn Tyr Ile Ile Ser Phe Thr Val Pro Asp Ile Asp Asn  
 1605 1610 1615  
 Val Thr Val Leu Asp Tyr Asp Ala Arg Glu Gln Arg Val Tyr Trp Ser  
 1620 1625 1630  
 Asp Val Arg Thr Gln Ala Ile Lys Arg Ala Phe Ile Asn Gly Thr Gly  
 1635 1640 1645  
 Val Glu Thr Val Val Ser Ala Asp Leu Pro Asn Ala His Gly Leu Ala  
 1650 1655 1660  
 Val Asp Trp Val Ser Arg Asn Leu Phe Trp Thr Ser Tyr Asp Thr Asn  
 665 1670 1675 1680  
 Lys Lys Gln Ile Asn Val Ala Arg Leu Asp Gly Ser Phe Lys Asn Ala  
 1685 1690 1695  
 Val Val Gln Gly Leu Glu Gln Pro His Gly Leu Val Val His Pro Leu  
 1700 1705 1710  
 Arg Gly Lys Leu Tyr Trp Thr Asp Gly Asp Asn Ile Ser Met Ala Asn  
 1715 1720 1725  
 Met Asp Gly Ser Asn Arg Thr Leu Leu Phe Ser Gly Gln Lys Gly Pro  
 1730 1735 1740  
 Val Gly Leu Ala Ile Asp Phe Pro Glu Ser Lys Leu Tyr Trp Ile Ser  
 745 1750 1755 1760  
 Ser Gly Asn His Thr Ile Asn Arg Cys Asn Leu Asp Gly Ser Gly Leu  
 1765 1770 1775  
 Glu Val Ile Asp Ala Met Arg Ser Gln Leu Gly Lys Ala Thr Ala Leu  
 1780 1785 1790  
 Ala Ile Met Gly Asp Lys Leu Trp Trp Ala Asp Gln Val Ser Glu Lys  
 1795 1800 1805  
 Met Gly Thr Cys Ser Lys Ala Asp Gly Ser Gly Ser Val Val Leu Arg  
 1810 1815 1820  
 Asn Ser Thr Thr Leu Val Met His Met Lys Val Tyr Asp Glu Ser Ile  
 825 1830 1835 1840  
 Gln Leu Asp His Lys Gly Thr Asn Pro Cys Ser Val Asn Asn Gly Asp  
 1845 1850 1855  
 Cys Ser Gln Leu Cys Leu Pro Thr Ser Glu Thr Thr Arg Ser Cys Met

FIG. 14B



Phe Val Leu Asp Glu Cys Gln Asn Leu Met Phe Trp Thr Asn Trp Asn  
 2340 2345 2350  
 Glu Gln His Pro Ser Ile Met Arg Ala Ala Leu Ser Gly Ala Asn Val  
 2355 2360 2365  
 Leu Thr Leu Ile Glu Lys Asp Ile Arg Thr Pro Asn Gly Leu Ala Ile  
 2370 2375 2380  
 Asp His Arg Ala Glu Lys Leu Tyr Phe Ser Asp Ala Thr Leu Asp Lys  
 385 2390 2395 2400  
 Ile Glu Arg Cys Glu Tyr Asp Gly Ser His Arg Tyr Val Ile Leu Lys  
 2405 2410 2415  
 Ser Glu Pro Val His Pro Phe Gly Leu Ala Val Tyr Gly Glu His Ile  
 2420 2425 2430  
 Phe Trp Thr Asp Trp Val Arg Arg Ala Val Gln Arg Ala Asn Lys His  
 2435 2440 2445  
 Val Gly Ser Asn Met Lys Leu Leu Arg Val Asp Ile Pro Gln Gln Pro  
 2450 2455 2460  
 Met Gly Ile Ile Ala Val Ala Asn Asp Thr Asn Ser Cys Glu Leu Ser  
 465 2470 2475 2480  
 Pro Cys Arg Ile Asn Asn Gly Gly Cys Gln Asp Leu Cys Leu Leu Thr  
 2485 2490 2495  
 His Gln Gly His Val Asn Cys Ser Cys Arg Gly Gly Arg Ile Leu Gln  
 2500 2505 2510  
 Asp Asp Leu Thr Cys Arg Ala Val Asn Ser Ser Cys Arg Ala Gln Asp  
 2515 2520 2525  
 Glu Phe Glu Cys Ala Asn Gly Glu Cys Ile Asn Phe Ser Leu Thr Cys  
 2530 2535 2540  
 Asp Gly Val Pro His Cys Lys Asp Lys Ser Asp Glu Lys Pro Ser Tyr  
 545 2550 2555 2560  
 Cys Asn Ser Arg Arg Cys Lys Lys Thr Phe Arg Gln Cys Ser Asn Gly  
 2565 2570 2575  
 Arg Cys Val Ser Asn Met Leu Trp Cys Asn Gly Ala Asp Asp Cys Gly  
 2580 2585 2590  
 Asp Gly Ser Asp Glu Ile Pro Cys Asn Lys Thr Ala Cys Gly Val Gly  
 2595 2600 2605  
 Glu Phe Arg Cys Arg Asp Gly Thr Cys Ile Gly Asn Ser Ser Arg Cys  
 2610 2615 2620  
 Asn Gln Phe Val Asp Cys Glu Asp Ala Ser Asp Glu Met Asn Cys Ser  
 625 2630 2635 2640  
 Ala Thr Asp Cys Ser Tyr Phe Arg Leu Gly Val Lys Gly Val Leu  
 2645 2650 2655  
 Phe Gln Pro Cys Glu Arg Thr Ser Leu Cys Tyr Ala Pro Ser Trp Val  
 2660 2665 2670  
 Cys Asp Gly Ala Asn Asp Cys Gly Asp Tyr Ser Asp Glu Arg Asp Cys  
 2675 2680 2685  
 Pro Gly Val Lys Arg Pro Arg Cys Pro Leu Asn Tyr Phe Ala Cys Pro  
 2690 2695 2700  
 Ser Gly Arg Cys Ile Pro Met Ser Trp Thr Cys Asp Lys Glu Asp Asp  
 705 2710 2715 2720  
 Cys Glu His Gly Glu Asp Glu Thr His Cys Asn Lys Phe Cys Ser Glu  
 2725 2730 2735  
 Ala Gln Phe Glu Cys Gln Asn His Arg Cys Ile Ser Lys Gln Trp Leu  
 2740 2745 2750  
 Cys Asp Gly Ser Asp Asp Cys Gly Asp Gly Ser Asp Glu Ala Ala His  
 2755 2760 2765  
 Cys Glu Gly Lys Thr Cys Gly Pro Ser Ser Phe Ser Cys Pro Gly Thr  
 2770 2775 2780  
 His Val Cys Val Pro Glu Arg Trp Leu Cys Asp Gly Asp Lys Asp Cys  
 785 2790 2795 2800  
 Ala Asp Gly Ala Asp Glu Ser Ile Ala Ala Gly Cys Leu Tyr Asn Ser

FIG. 14B



Phe His Ala Leu Arg Gln Pro Asp Val Pro Asn His Pro Cys Lys Val  
 3285 3290 3295  
 Asn Asn Gly Gly Cys Ser Asn Leu Cys Leu Leu Ser Pro Gly Gly Gly  
 3300 3305 3310  
 His Lys Cys Ala Cys Pro Thr Asn Phe Tyr Leu Gly Ser Asp Gly Arg  
 3315 3320 3325  
 Thr Cys Val Ser Asn Cys Thr Ala Ser Gln Phe Val Cys Lys Asn Asp  
 3330 3335 3340  
 Lys Cys Ile Pro Phe Trp Trp Lys Cys Asp Thr Glu Asp Asp Cys Gly  
 345 3350 3355 3360  
 Asp His Ser Asp Glu Pro Pro Asp Cys Pro Glu Phe Lys Cys Arg Pro  
 3365 3370 3375  
 Gly Gln Phe Gln Cys Ser Thr Gly Ile Cys Thr Asn Pro Ala Phe Ile  
 3380 3385 3390  
 Cys Asp Gly Asp Asn Asp Cys Gln Asp Asn Ser Asp Glu Ala Asn Cys  
 3395 3400 3405  
 Asp Ile His Val Cys Leu Pro Ser Gln Phe Lys Cys Thr Asn Thr Asn  
 3410 3415 3420  
 Arg Cys Ile Pro Gly Ile Phe Arg Cys Asn Gly Gln Asp Asn Cys Gly  
 425 3430 3435 3440  
 Asp Gly Glu Asp Glu Arg Asp Cys Pro Glu Val Thr Cys Ala Pro Asn  
 3445 3450 3455  
 Gln Phe Gln Cys Ser Ile Thr Lys Arg Cys Ile Pro Arg Val Trp Val  
 3460 3465 3470  
 Cys Asp Arg Asp Asn Asp Cys Val Asp Gly Ser Asp Glu Pro Ala Asn  
 3475 3480 3485  
 Cys Thr Gln Met Thr Cys Gly Val Asp Glu Phe Arg Cys Lys Asp Ser  
 3490 3495 3500  
 Gly Arg Cys Ile Pro Ala Arg Trp Lys Cys Asp Gly Glu Asp Asp Cys  
 505 3510 3515 3520  
 Gly Asp Gly Ser Asp Glu Pro Lys Glu Glu Cys Asp Glu Arg Thr Cys  
 3525 3530 3535  
 Glu Pro Tyr Gln Phe Arg Cys Lys Asn Asn Arg Cys Val Pro Gly Arg  
 3540 3545 3550  
 Trp Gln Cys Asp Tyr Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Glu  
 3555 3560 3565  
 Ser Cys Thr Pro Arg Pro Cys Ser Glu Ser Glu Phe Ser Cys Ala Asn  
 3570 3575 3580  
 Gly Arg Cys Ile Ala Gly Arg Trp Lys Cys Asp Gly Asp His Asp Cys  
 585 3590 3595 3600  
 Ala Asp Gly Ser Asp Glu Lys Asp Cys Thr Pro Arg Cys Asp Met Asp  
 3605 3610 3615  
 Gln Phe Gln Cys Lys Ser Gly His Cys Ile Pro Leu Arg Trp Arg Cys  
 3620 3625 3630  
 Asp Ala Asp Ala Asp Cys Met Asp Gly Ser Asp Glu Glu Ala Cys Gly  
 3635 3640 3645  
 Thr Gly Val Arg Thr Cys Pro Leu Asp Glu Phe Gln Cys Asn Asn Thr  
 3650 3655 3660  
 Leu Cys Lys Pro Leu Ala Trp Lys Cys Asp Gly Glu Asp Asp Cys Gly  
 665 3670 3675 3680  
 Asp Asn Ser Asp Glu Asn Pro Glu Glu Cys Ala Arg Phe Val Cys Pro  
 3685 3690 3695  
 Pro Asn Arg Pro Phe Arg Cys Lys Asn Asp Arg Val Cys Leu Trp Ile  
 3700 3705 3710  
 Gly Arg Gln Cys Asp Gly Thr Asp Asn Cys Gly Asp Gly Thr Asp Glu  
 3715 3720 3725  
 Glu Asp Cys Glu Pro Pro Thr Ala His Thr Thr His Cys Lys Asp Lys  
 3730 3735 3740  
 Lys Glu Phe Leu Cys Arg Asn Gln Arg Cys Leu Ser Ser Ser Leu Arg

FIG. 14B

745 3750 3755 3760  
 Cys Asn Met Phe Asp Asp Cys Gly Asp Gly Ser Asp Glu Glu Asp Cys  
 Ser Ile Asp Pro Lys Leu Thr Ser Cys Ala Thr Asn Ala Ser Ile Cys  
 Gly Asp Glu Ala Arg Cys Val Arg Thr Glu Lys Ala Ala Tyr Cys Ala  
 Cys Arg Ser Gly Phe His Thr Val Pro Gly Gln Pro Gly Cys Gln Asp  
 Ile Asn Glu Cys Leu Arg Phe Gly Thr Cys Ser Gln Leu Cys Asn Asn  
 Thr Lys Gly Gly His Leu Cys Ser Cys Ala Arg Asn Phe Met Lys Thr  
 His Asn Thr Cys Lys Ala Glu Gly Ser Glu Tyr Gln Val Leu Tyr Ile  
 Ala Asp Asp Asn Glu Ile Arg Ser Leu Phe Pro Gly His Pro His Ser  
 Ala Tyr Glu Gln Ala Phe Gln Gly Asp Glu Ser Val Arg Ile Asp Ala  
 Met Asp Val His Val Lys Ala Gly Arg Val Tyr Trp Thr Asn Trp His  
 Thr Gly Thr Ile Ser Tyr Arg Ser Leu Pro Pro Ala Ala Pro Pro Thr  
 Thr Ser Asn Arg His Arg Arg Gln Ile Asp Arg Gly Val Thr His Leu  
 Asn Ile Ser Gly Leu Lys Met Pro Arg Gly Ile Ala Ile Asp Trp Val  
 Ala Gly Asn Val Tyr Trp Thr Asp Ser Gly Arg Asp Val Ile Glu Val  
 Ala Gln Met Lys Gly Glu Asn Arg Lys Thr Leu Ile Ser Gly Met Ile  
 Asp Glu Pro His Ala Ile Val Val Asp Pro Leu Arg Gly Thr Met Tyr  
 Trp Ser Asp Trp Gly Asn His Pro Lys Ile Glu Thr Ala Ala Met Asp  
 Gly Thr Leu Arg Glu Thr Leu Val Gln Asp Asn Ile Gln Trp Pro Thr  
 Gly Leu Ala Val Asp Tyr His Asn Glu Arg Leu Tyr Trp Ala Asp Ala  
 Lys Leu Ser Val Ile Gly Ser Ile Arg Leu Asn Gly Thr Asp Pro Ile  
 Val Ala Ala Asp Ser Lys Arg Gly Leu Ser His Pro Phe Ser Ile Asp  
 Val Phe Glu Asp Tyr Ile Tyr Gly Val Thr Tyr Ile Asn Asn Arg Val  
 Phe Lys Ile His Lys Phe Gly His Ser Pro Leu Val Asn Leu Thr Gly  
 Gly Leu Ser His Ala Ser Asp Val Val Leu Tyr His Gln His Lys Gln  
 Pro Glu Val Thr Asn Pro Cys Asp Arg Lys Lys Cys Glu Trp Leu Cys  
 Leu Leu Ser Pro Ser Gly Pro Val Cys Thr Cys Pro Asn Gly Lys Arg  
 Leu Asp Asn Gly Thr Cys Val Pro Val Pro Ser Pro Thr Pro Pro  
 Asp Ala Pro Arg Pro Gly Thr Cys Asn Leu Gln Cys Phe Asn Gly Gly  
 Ser Cys Phe Leu Asn Ala Arg Arg Gln Pro Lys Cys Arg Cys Gln Pro

FIG. 14B

Arg Tyr Thr Gly Asp Lys Cys Glu Leu Asp Gln Cys Trp Glu His Cys  
 225 4230 4235 4240  
 Arg Asn Gly Gly Thr Cys Ala Ala Ser Pro Ser Gly Met Pro Thr Cys  
 4245 4250 4255  
 Arg Cys Pro Thr Gly Phe Thr Gly Pro Lys Cys Thr Gln Gln Val Cys  
 4260 4265 4270  
 Ala Gly Tyr Cys Ala Asn Asn Ser Thr Cys Thr Val Asn Gln Gly Asn  
 4275 4280 4285  
 Gln Pro Gln Cys Arg Cys Leu Pro Gly Phe Leu Gly Asp Arg Cys Gln  
 4290 4295 4300  
 Tyr Arg Gln Cys Ser Gly Tyr Cys Glu Asn Phe Gly Thr Cys Gln Met  
 305 4310 4315 4320  
 Ala Ala Asp Gly Ser Arg Gln Cys Arg Cys Thr Ala Tyr Phe Glu Gly  
 4325 4330 4335  
 Ser Arg Cys Glu Val Asn Lys Cys Ser Arg Cys Leu Glu Gly Ala Cys  
 4340 4345 4350  
 Val Val Asn Lys Gln Ser Gly Asp Val Thr Cys Asn Cys Thr Asp Gly  
 4355 4360 4365  
 Arg Val Ala Pro Ser Cys Leu Thr Cys Val Gly His Cys Ser Asn Gly  
 4370 4375 4380  
 Gly Ser Cys Thr Met Asn Ser Lys Met Met Pro Glu Cys Gln Cys Pro  
 385 4390 4395 4400  
 Pro His Met Thr Gly Pro Arg Cys Glu Glu His Val Phe Ser Gln Gln  
 4405 4410 4415  
 Gln Pro Gly His Ile Ala Ser Ile Leu Ile Pro Leu Leu Leu Leu Leu  
 4420 4425 4430  
 Leu Leu Val Leu Val Ala Gly Val Val Phe Trp Tyr Lys Arg Arg Val  
 4435 4440 4445  
 Gln Gly Ala Lys Gly Phe Gln His Gln Arg Met Thr Asn Gly Ala Met  
 4450 4455 4460  
 Asn Val Glu Ile Gly Asn Pro Thr Tyr Lys Met Tyr Glu Gly Gly Glu  
 465 4470 4475 4480  
 Pro Asp Asp Val Gly Gly Leu Leu Asp Ala Asp Phe Ala Leu Asp Pro  
 4485 4490 4495  
 Asp Lys Pro Thr Asn Phe Thr Asn Pro Val Tyr Ala Thr Leu Tyr Met  
 4500 4505 4510  
 Gly Gly His Gly Ser Arg His Ser Leu Ala Ser Thr Asp Glu Lys Arg  
 4515 4520 4525  
 Glu Leu Leu Gly Arg Gly Pro Glu Asp Glu Ile Gly Asp Pro Leu Ala  
 4530 4535 4540

FIG. 14B

SEQUENCE LISTING

<110> Antigenics, Inc.

<120> ALPHA(2) MACROGLOBULIN RECEPTOR AS A HEAT SHOCK  
PROTEIN RECEPTOR AND USES THEREOF

<130> 8449-134-228

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<150> 09/750,972

<151> 2000-12-28

<150> 09/668,724

<151> 2000-09-22

<150> 60/209,095

<151> 2000-06/02

<160> 57

<170> FastSEQ for Windows Version 3.0

<210> 1

<211> 14849

<212> DNA

<213> Mus musculus

<400> 1

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<210> 7
<211> 126
<212> PRT
<213> Homo sapiens

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<400> 7
Ile Ala Leu Asp Phe His Leu Ser Gln Ser Ala Leu Tyr Trp Thr Asp
 1                    5                    10                    15
Val Val Glu Asp Lys Ile Tyr Arg Gly Lys Leu Leu Asp Asn Gly Ala
                20                    25                    30
Leu Thr Ser Phe Glu Val Val Ile Gln Tyr Gly Leu Ala Thr Pro Glu
                35                    40                    45
Gly Leu Ala Val Asp Trp Ile Ala Gly Asn Ile Tyr Trp Val Glu Ser
                50                    55                    60
Asn Leu Asp Gln Ile Glu Val Ala Lys Leu Asp Gly Thr Leu Arg Thr
        65                    70                    75                    80
Thr Leu Leu Ala Gly Asp Ile Glu His Pro Arg Ala Ile Ala Leu Asp
                85                    90                    95
Pro Arg Asp Gly Ile Leu Phe Trp Thr Asp Trp Asp Ala Ser Leu Pro
                100                    105                    110
Arg Ile Glu Ala Ala Ser Met Ser Gly Ala Gly Arg Arg Thr
                115                    120                    125

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<210> 8
<211> 153
<212> PRT
<213> Homo sapiens

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<400> 8
Leu Leu Gln Gln Val Ser Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met
 1                    5                    10                    15
Lys Val Thr Gly Glu Gly Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr

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20 25 30  
 Asn Ile Leu Pro Glu Lys Glu Glu Phe Pro Phe Ala Leu Gly Val Gln  
 35 40 45  
 Thr Leu Pro Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln  
 50 55 60  
 Ile Ser Leu Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met  
 65 70 75 80  
 Ala Ile Val Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro  
 85 90 95  
 Thr Val Lys Met Leu Glu Arg Ser Asn His Val Ser Arg Thr Glu Val  
 100 105 110  
 Ser Ser Asn His Val Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln Thr  
 115 120 125  
 Leu Ser Leu Phe Phe Thr Val Leu Gln Asp Val Pro Val Arg Asp Leu  
 130 135 140  
 Lys Pro Ala Ile Val Lys Val Tyr Asp  
 145 150

<210> 9  
 <211> 138  
 <212> PRT  
 <213> Homo sapiens

<400> 9  
 Met Lys Val Thr Gly Glu Gly Cys Val Tyr Leu Gln Thr Ser Leu Lys  
 1 5 10 15  
 Tyr Asn Ile Leu Pro Glu Lys Glu Glu Phe Pro Phe Ala Leu Gly Val  
 20 25 30  
 Gln Thr Leu Pro Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe  
 35 40 45  
 Gln Ile Ser Leu Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn  
 50 55 60  
 Met Ala Ile Val Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu Lys  
 65 70 75 80  
 Pro Thr Val Lys Met Leu Glu Arg Ser Asn His Val Ser Arg Thr Glu  
 85 90 95  
 Val Ser Ser Asn His Val Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln  
 100 105 110  
 Thr Leu Ser Leu Phe Phe Thr Val Leu Gln Asp Val Pro Val Arg Asp  
 115 120 125  
 Leu Lys Pro Ala Ile Val Lys Val Tyr Asp  
 130 135

<210> 10  
 <211> 27  
 <212> PRT  
 <213> Homo sapiens

<400> 10  
 Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile Val  
 1 5 10 15  
 Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu  
 20 25

<210> 11  
 <211> 126  
 <212> PRT  
 <213> Homo sapiens

<400> 11  
 Leu Gln Gln Val Ser Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys  
 1 5 10 15  
 Val Thr Gly Glu Gly Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn  
 20 25 30  
 Ile Leu Pro Glu Lys Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr  
 35 40 45  
 Leu Pro Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile  
 50 55 60  
 Ser Leu Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala  
 65 70 75 80  
 Ile Val Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr  
 85 90 95  
 Val Lys Met Leu Glu Arg Ser Asn His Val Ser Arg Thr Glu Val Ser  
 100 105 110  
 Ser Asn His Val Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln  
 115 120 125

<210> 12  
 <211> 111  
 <212> PRT  
 <213> Homo sapiens

<400> 12  
 Leu Gln Gln Val Ser Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys  
 1 5 10 15  
 Val Thr Gly Glu Gly Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn  
 20 25 30  
 Ile Leu Pro Glu Lys Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr  
 35 40 45  
 Leu Pro Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile  
 50 55 60  
 Ser Leu Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala  
 65 70 75 80  
 Ile Val Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr  
 85 90 95  
 Val Lys Met Leu Glu Arg Ser Asn His Val Ser Arg Thr Glu Val  
 100 105 110

<210> 13  
 <211> 81  
 <212> PRT  
 <213> Homo sapiens

<400> 13  
 Leu Gln Gln Val Ser Leu Pro Glu Leu Pro Gly Glu Tyr Ser Met Lys  
 1 5 10 15  
 Val Thr Gly Glu Gly Cys Val Tyr Leu Gln Thr Ser Leu Lys Tyr Asn  
 20 25 30  
 Ile Leu Pro Glu Lys Glu Glu Phe Pro Phe Ala Leu Gly Val Gln Thr  
 35 40 45  
 Leu Pro Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile  
 50 55 60  
 Ser Leu Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala  
 65 70 75 80  
 Ile

<210> 14  
 <211> 101  
 <212> PRT  
 <213> Homo sapiens

<400> 14  
 Gln Thr Ser Leu Lys Tyr Asn Ile Leu Pro Glu Lys Glu Glu Phe Pro  
 1 5 10 15  
 Phe Ala Leu Gly Val Gln Thr Leu Pro Gln Thr Cys Asp Glu Pro Lys  
 20 25 30  
 Ala His Thr Ser Phe Gln Ile Ser Leu Ser Val Ser Tyr Thr Gly Ser  
 35 40 45  
 Arg Ser Ala Ser Asn Met Ala Ile Val Asp Val Lys Met Val Ser Gly  
 50 55 60  
 Phe Ile Pro Leu Lys Pro Thr Val Lys Met Leu Glu Arg Ser Asn His  
 65 70 75 80  
 Val Ser Arg Thr Glu Val Ser Ser Asn His Val Leu Ile Tyr Leu Asp  
 85 90 95  
 Lys Val Ser Asn Gln  
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<210> 15  
 <211> 76  
 <212> PRT  
 <213> Homo sapiens

<400> 15  
 Gln Thr Ser Leu Lys Tyr Asn Ile Leu Pro Glu Lys Glu Glu Phe Pro  
 1 5 10 15  
 Phe Ala Leu Gly Val Gln Thr Leu Pro Gln Thr Cys Asp Glu Pro Lys  
 20 25 30  
 Ala His Thr Ser Phe Gln Ile Ser Leu Ser Val Ser Tyr Thr Gly Ser  
 35 40 45  
 Arg Ser Ala Ser Asn Met Ala Ile Val Asp Val Lys Met Val Ser Gly  
 50 55 60  
 Phe Ile Pro Leu Lys Pro Thr Val Lys Met Leu Glu  
 65 70 75

<210> 16  
 <211> 56  
 <212> PRT  
 <213> Homo sapiens

<400> 16  
 Gln Thr Ser Leu Lys Tyr Asn Ile Leu Pro Glu Lys Glu Glu Phe Pro  
 1 5 10 15  
 Phe Ala Leu Gly Val Gln Thr Leu Pro Gln Thr Cys Asp Glu Pro Lys  
 20 25 30  
 Ala His Thr Ser Phe Gln Ile Ser Leu Ser Val Ser Tyr Thr Gly Ser  
 35 40 45  
 Arg Ser Ala Ser Asn Met Ala Ile  
 50 55

<210> 17  
 <211> 76  
 <212> PRT  
 <213> Homo sapiens

<400> 17  
 Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile Ser Leu  
 1 5 10 15  
 Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile Val  
 20 25 30  
 Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val Lys  
 35 40 45  
 Met Leu Glu Arg Ser Asn His Val Ser Arg Thr Glu Val Ser Ser Asn  
 50 55 60  
 His Val Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln  
 65 70 75

<210> 18  
 <211> 76  
 <212> PRT  
 <213> Homo sapiens

<400> 18  
 Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile Ser Leu  
 1 5 10 15  
 Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile Val  
 20 25 30  
 Asp Val Lys Met Val Ser Gly Phe Ile Pro Leu Lys Pro Thr Val Lys  
 35 40 45  
 Met Leu Glu Arg Ser Asn His Val Ser Arg Thr Glu Val Ser Ser Asn  
 50 55 60  
 His Val Leu Ile Tyr Leu Asp Lys Val Ser Asn Gln  
 65 70 75

<210> 19  
 <211> 31  
 <212> PRT  
 <213> Homo sapiens

<400> 19  
 Gln Thr Cys Asp Glu Pro Lys Ala His Thr Ser Phe Gln Ile Ser Leu  
 1 5 10 15  
 Ser Val Ser Tyr Thr Gly Ser Arg Ser Ala Ser Asn Met Ala Ile  
 20 25 30

<210> 20  
 <211> 44  
 <212> PRT  
 <213> Homo sapiens

<400> 20  
 Lys Thr Cys Ser Pro Lys Gln Phe Ala Cys Arg Asp Gln Ile Thr Cys  
 1 5 10 15  
 Ile Ser Lys Gly Trp Arg Cys Asp Gly Glu Arg Asp Cys Pro Asp Gly  
 20 25 30  
 Ser Asp Glu Ala Pro Glu Ile Cys Pro Gln Ser Lys  
 35 40

<210> 21  
 <211> 86  
 <212> PRT  
 <213> Homo sapiens

<400> 21  
 Lys Thr Cys Ser Pro Lys Gln Phe Ala Cys Arg Asp Gln Ile Thr Cys  
 1 5 10 15  
 Ile Ser Lys Gly Trp Arg Cys Asp Gly Glu Arg Asp Cys Pro Asp Gly  
 20 25 30  
 Ser Asp Glu Ala Pro Glu Ile Cys Pro Gln Ser Lys Ala Gln Arg Cys  
 35 40 45  
 Gln Pro Asn Glu His Asn Cys Leu Gly Thr Glu Leu Cys Val Pro Met  
 50 55 60  
 Ser Arg Leu Cys Asn Gly Val Gln Asp Cys Met Asp Gly Ser Asp Glu  
 65 70 75 80  
 Gly Pro His Cys Arg Glu  
 85

<210> 22  
 <211> 43  
 <212> PRT  
 <213> Homo sapiens

<400> 22  
 Lys Ala Gln Arg Cys Gln Pro Asn Glu His Asn Cys Leu Gly Thr Glu  
 1 5 10 15  
 Leu Cys Val Pro Met Ser Arg Leu Cys Asn Gly Val Gln Asp Cys Met  
 20 25 30  
 Asp Gly Ser Asp Glu Gly Pro His Cys Arg Glu  
 35 40

<210> 23  
 <211> 42  
 <212> PRT  
 <213> Homo sapiens

<400> 23  
 Gln Cys Gln Pro Gly Glu Phe Ala Cys Ala Asn Ser Arg Cys Ile Gln  
 1 5 10 15  
 Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys Leu Asp Asn Ser Asp  
 20 25 30  
 Glu Ala Pro Ala Leu Cys His Gln His Thr  
 35 40

<210> 24  
 <211> 82  
 <212> PRT  
 <213> Homo sapiens

<400> 24  
 Gln Cys Gln Pro Gly Glu Phe Ala Cys Ala Asn Ser Arg Cys Ile Gln  
 1 5 10 15  
 Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys Leu Asp Asn Ser Asp  
 20 25 30  
 Glu Ala Pro Ala Leu Cys His Gln His Thr Cys Pro Ser Asp Arg Phe  
 35 40 45  
 Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg Trp Leu Cys Asp Gly  
 50 55 60  
 Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys Ser  
 65 70 75 80  
 Ala Arg

<210> 25  
 <211> 122  
 <212> PRT  
 <213> Homo sapiens

<400> 25  
 Gln Cys Gln Pro Gly Glu Phe Ala Cys Ala Asn Ser Arg Cys Ile Gln  
 1 5 10 15  
 Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys Leu Asp Asn Ser Asp  
 20 25 30  
 Glu Ala Pro Ala Leu Cys His Gln His Thr Cys Pro Ser Asp Arg Phe  
 35 40 45  
 Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg Trp Leu Cys Asp Gly  
 50 55 60  
 Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys Ser  
 65 70 75 80  
 Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg Cys  
 85 90 95  
 Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp Asp Cys Gly Asp Arg  
 100 105 110  
 Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro  
 115 120

<210> 26  
 <211> 161  
 <212> PRT  
 <213> Homo sapiens

<400> 26  
 Gln Cys Gln Pro Gly Glu Phe Ala Cys Ala Asn Ser Arg Cys Ile Gln  
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 Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys Leu Asp Asn Ser Asp  
 20 25 30  
 Glu Ala Pro Ala Leu Cys His Gln His Thr Cys Pro Ser Asp Arg Phe  
 35 40 45  
 Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg Trp Leu Cys Asp Gly  
 50 55 60  
 Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys Ser  
 65 70 75 80  
 Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg Cys  
 85 90 95  
 Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp Asp Cys Gly Asp Arg  
 100 105 110  
 Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu Thr  
 115 120 125  
 Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg Cys  
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 Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Ala Gly Cys Ser  
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 His

<210> 27  
 <211> 208  
 <212> PRT  
 <213> Homo sapiens

<400> 27

Gln Cys Gln Pro Gly Glu Phe Ala Cys Ala Asn Ser Arg Cys Ile Gln  
 1 5 10 15  
 Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys Leu Asp Asn Ser Asp  
 20 25 30  
 Glu Ala Pro Ala Leu Cys His Gln His Thr Cys Pro Ser Asp Arg Phe  
 35 40 45  
 Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg Trp Leu Cys Asp Gly  
 50 55 60  
 Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys Ser  
 65 70 75 80  
 Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg Cys  
 85 90 95  
 Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp Asp Cys Gly Asp Arg  
 100 105 110  
 Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu Thr  
 115 120 125  
 Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg Cys  
 130 135 140  
 Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Ala Gly Cys Ser  
 145 150 155 160  
 His Ser Cys Ser Ser Thr Gln Phe Lys Cys Asn Ser Gly Arg Cys Ile  
 165 170 175  
 Pro Glu His Trp Thr Cys Asp Gly Asp Asn Asp Cys Gly Asp Tyr Ser  
 180 185 190  
 Asp Glu Thr His Ala Asn Cys Thr Asn Gln Ala Thr Arg Pro Pro Gly  
 195 200 205

<210> 28  
 <211> 150  
 <212> PRT  
 <213> Homo sapiens

<400> 28  
 Gln Cys Gln Pro Gly Glu Phe Ala Cys Ala Asn Ser Arg Cys Ile Gln  
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 Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys Leu Asp Asn Ser Asp  
 20 25 30  
 Glu Ala Pro Ala Leu Cys His Gln His Thr Cys Pro Ser Asp Arg Phe  
 35 40 45  
 Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg Trp Leu Cys Asp Gly  
 50 55 60  
 Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys Ser  
 65 70 75 80  
 Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg Cys  
 85 90 95  
 Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp Asp Cys Gly Asp Arg  
 100 105 110  
 Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu Thr  
 115 120 125  
 Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg Cys  
 130 135 140  
 Asp Asn Asp Asn Asp Cys  
 145 150

<210> 29  
 <211> 231  
 <212> PRT  
 <213> Homo sapiens

<400> 29  
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 1 5 10 15  
 Glu Arg Trp Lys Cys Asp Gly Asp Asn Asp Cys Leu Asp Asn Ser Asp  
 20 25 30  
 Glu Ala Pro Ala Leu Cys His Gln His Thr Cys Pro Ser Asp Arg Phe  
 35 40 45  
 Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn Arg Trp Leu Cys Asp Gly  
 50 55 60  
 Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu Ser Asn Ala Thr Cys Ser  
 65 70 75 80  
 Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg Cys  
 85 90 95  
 Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp Asp Asp Cys Gly Asp Arg  
 100 105 110  
 Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu Thr  
 115 120 125  
 Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg Cys  
 130 135 140  
 Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Ala Gly Cys Ser  
 145 150 155 160  
 His Ser Cys Ser Ser Thr Gln Phe Lys Cys Asn Ser Gly Arg Cys Ile  
 165 170 175  
 Pro Glu His Trp Thr Cys Asp Gly Asp Asn Asp Cys Gly Asp Tyr Ser  
 180 185 190  
 Asp Glu Thr His Ala Asn Cys Thr Asn Gln Ala Thr Arg Pro Pro Gly  
 195 200 205  
 Gly Cys His Thr Asp Glu Phe Gln Cys Arg Leu Asp Gly Leu Cys Ile  
 210 215 220  
 Pro Leu Arg Trp Arg Cys Asp  
 225 230

<210> 30  
 <211> 40  
 <212> PRT  
 <213> Homo sapiens

<400> 30  
 Cys Pro Ser Asp Arg Phe Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn  
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 Arg Trp Leu Cys Asp Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu  
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 Ser Asn Ala Thr Cys Ser Ala Arg  
 35 40

<210> 31  
 <211> 80  
 <212> PRT  
 <213> Homo sapiens

<400> 31  
 Cys Pro Ser Asp Arg Phe Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn  
 1 5 10 15  
 Arg Trp Leu Cys Asp Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu  
 20 25 30  
 Ser Asn Ala Thr Cys Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser  
 35 40 45

Cys Ala Ser Gly Arg Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp  
 50 55 60  
 Asp Asp Cys Gly Asp Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro  
 65 70 75 80

<210> 32  
 <211> 119  
 <212> PRT  
 <213> Homo sapiens

<400> 32  
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 Arg Trp Leu Cys Asp Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu  
 20 25 30  
 Ser Asn Ala Thr Cys Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser  
 35 40 45  
 Cys Ala Ser Gly Arg Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp  
 50 55 60  
 Asp Asp Cys Gly Asp Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro  
 65 70 75 80  
 Thr Cys Phe Pro Leu Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile  
 85 90 95  
 Asn Ile Asn Trp Arg Cys Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser  
 100 105 110  
 Asp Glu Ala Gly Cys Ser His  
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<210> 33  
 <211> 166  
 <212> PRT  
 <213> Homo sapiens

<400> 33  
 Cys Pro Ser Asp Arg Phe Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn  
 1 5 10 15  
 Arg Trp Leu Cys Asp Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu  
 20 25 30  
 Ser Asn Ala Thr Cys Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser  
 35 40 45  
 Cys Ala Ser Gly Arg Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp  
 50 55 60  
 Asp Asp Cys Gly Asp Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro  
 65 70 75 80  
 Thr Cys Phe Pro Leu Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile  
 85 90 95  
 Asn Ile Asn Trp Arg Cys Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser  
 100 105 110  
 Asp Glu Ala Gly Cys Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys  
 115 120 125  
 Asn Ser Gly Arg Cys Ile Pro Glu His Trp Thr Cys Asp Gly Asp Asn  
 130 135 140  
 Asp Cys Gly Asp Tyr Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln  
 145 150 155 160  
 Ala Thr Arg Pro Pro Gly  
 165

<210> 34

<211> 108  
 <212> PRT  
 <213> Homo sapiens

<400> 34  
 Cys Pro Ser Asp Arg Phe Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn  
 1 5 10 15  
 Arg Trp Leu Cys Asp Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu  
 20 25 30  
 Ser Asn Ala Thr Cys Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser  
 35 40 45  
 Cys Ala Ser Gly Arg Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp  
 50 55 60  
 Asp Asp Cys Gly Asp Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro  
 65 70 75 80  
 Thr Cys Phe Pro Leu Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile  
 85 90 95  
 Asn Ile Asn Trp Arg Cys Asp Asn Asp Asn Asp Cys  
 100 105

<210> 35  
 <211> 289  
 <212> PRT  
 <213> Homo sapiens

<400> 35  
 Cys Pro Ser Asp Arg Phe Lys Cys Glu Asn Asn Arg Cys Ile Pro Asn  
 1 5 10 15  
 Arg Trp Leu Cys Asp Gly Asp Asn Asp Cys Gly Asn Ser Glu Asp Glu  
 20 25 30  
 Ser Asn Ala Thr Cys Ser Ala Arg Thr Cys Pro Pro Asn Gln Phe Ser  
 35 40 45  
 Cys Ala Ser Gly Arg Cys Ile Pro Ile Ser Trp Thr Cys Asp Leu Asp  
 50 55 60  
 Asp Asp Cys Gly Asp Arg Ser Asp Glu Ser Ala Ser Cys Ala Tyr Pro  
 65 70 75 80  
 Thr Cys Phe Pro Leu Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile  
 85 90 95  
 Asn Ile Asn Trp Arg Cys Asp Asn Asp Asn Asp Cys Gly Asp Asn Ser  
 100 105 110  
 Asp Glu Ala Gly Cys Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys  
 115 120 125  
 Asn Ser Gly Arg Cys Ile Pro Glu His Trp Thr Cys Asp Gly Asp Asn  
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 Asp Cys Gly Asp Tyr Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln  
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 Ala Thr Arg Pro Pro Gly Gly Cys His Thr Asp Glu Phe Gln Cys Arg  
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 Leu Asp Gly Leu Cys Ile Pro Leu Arg Trp Arg Cys Asp Gly Asp Thr  
 180 185 190  
 Asp Cys Met Asp Ser Ser Asp Glu Lys Ser Cys Glu Gly Val Thr His  
 195 200 205  
 Val Cys Asp Pro Ser Val Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys  
 210 215 220  
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 225 230 235 240  
 Ser Asp Glu Glu Asn Cys Glu Ser Leu Ala Cys Arg Pro Pro Ser His  
 245 250 255

Pro Cys Ala Asn Asn Thr Ser Val Cys Leu Pro Pro Asp Lys Leu Cys  
 260 265 270  
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 275 280 285  
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 <212> PRT  
 <213> Homo sapiens

<400> 36  
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<210> 37  
 <211> 79  
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<400> 37  
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 20 25 30  
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 35 40 45  
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<400> 38  
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 Glu Ser Ala Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu Thr Gln Phe  
 35 40 45  
 Thr Cys Asn Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg Cys Asp Asn  
 50 55 60  
 Asp Asn Asp Cys Gly Asp Asn Ser Asp Glu Ala Gly Cys Ser His Ser  
 65 70 75 80  
 Cys Ser Ser Thr Gln Phe Lys Cys Asn Ser Gly Arg Cys Ile Pro Glu  
 85 90 95  
 His Trp Thr Cys Asp Gly Asp Asn Asp Cys Gly Asp Tyr Ser Asp Glu  
 100 105 110

Thr His Ala Asn Cys Thr Asn Gln Ala Thr Arg Pro Pro Gly  
 115 120 125

<210> 39  
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 <212> PRT  
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<400> 39

Thr Cys Pro Pro Asn Gln Phe Ser Cys Ala Ser Gly Arg Cys Ile Pro  
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 Asp Asn Asp Cys  
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<210> 40  
 <211> 248  
 <212> PRT  
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<400> 40

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 Ser Ala Ser Cys Ala Tyr Pro Thr Cys Phe Pro Leu Thr Gln Phe Thr  
 35 40 45  
 Cys Asn Asn Gly Arg Cys Ile Asn Ile Asn Trp Arg Cys Asp Asn Asp  
 50 55 60  
 Asn Asp Cys Gly Asp Asn Ser Asp Glu Ala Gly Cys Ser His Ser Cys  
 65 70 75 80  
 Ser Ser Thr Gln Phe Lys Cys Asn Ser Gly Arg Cys Ile Pro Glu His  
 85 90 95  
 Trp Thr Cys Asp Gly Asp Asn Asp Cys Gly Asp Tyr Ser Asp Glu Thr  
 100 105 110  
 His Ala Asn Cys Thr Asn Gln Ala Thr Arg Pro Pro Gly Gly Cys His  
 115 120 125  
 Thr Asp Glu Phe Gln Cys Arg Leu Asp Gly Leu Cys Ile Pro Leu Arg  
 130 135 140  
 Trp Arg Cys Asp Gly Asp Thr Asp Cys Met Asp Ser Ser Asp Glu Lys  
 145 150 155 160  
 Ser Cys Glu Gly Val Thr His Val Cys Asp Pro Ser Val Lys Phe Gly  
 165 170 175  
 Cys Lys Asp Ser Ala Arg Cys Ile Ser Lys Ala Trp Val Cys Asp Gly  
 180 185 190  
 Asp Asn Asp Cys Glu Asp Asn Ser Asp Glu Glu Asn Cys Glu Ser Leu  
 195 200 205  
 Ala Cys Arg Pro Pro Ser His Pro Cys Ala Asn Asn Thr Ser Val Cys  
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 225 230 235 240  
 Ser Asp Glu Gly Glu Leu Cys Asp  
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<210> 41  
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<210> 42  
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<400> 42  
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 35 40 45  
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 Ala Thr Arg Pro Pro Gly  
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<210> 43  
 <211> 169  
 <212> PRT  
 <213> Homo sapiens

<400> 43  
 Thr Cys Phe Pro Leu Thr Gln Phe Thr Cys Asn Asn Gly Arg Cys Ile  
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 20 25 30  
 Asp Glu Ala Gly Cys Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys  
 35 40 45  
 Asn Ser Gly Arg Cys Ile Pro Glu His Trp Thr Cys Asp Gly Asp Asn  
 50 55 60  
 Asp Cys Gly Asp Tyr Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln  
 65 70 75 80  
 Ala Thr Arg Pro Pro Gly Gly Cys His Thr Asp Glu Phe Gln Cys Arg  
 85 90 95  
 Leu Asp Gly Leu Cys Ile Pro Leu Arg Trp Arg Cys Asp Gly Asp Thr  
 100 105 110  
 Asp Cys Met Asp Ser Ser Asp Glu Lys Ser Cys Glu Gly Val Thr His  
 115 120 125  
 Val Cys Asp Pro Ser Val Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys  
 130 135 140  
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<210> 44  
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20 25 30  
Asp Glu Ala Gly Cys Ser His Ser Cys Ser Ser Thr Gln Phe Lys Cys  
35 40 45  
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50 55 60  
Asp Cys Gly Asp Tyr Ser Asp Glu Thr His Ala Asn Cys Thr Asn Gln  
65 70 75 80  
Ala Thr Arg Pro Pro Gly Gly Cys His Thr Asp Glu Phe Gln Cys Arg  
85 90 95  
Leu Asp Gly Leu Cys Ile Pro Leu Arg Trp Arg Cys Asp Gly Asp Thr  
100 105 110  
Asp Cys Met Asp Ser Ser Asp Glu Lys Ser Cys Glu Gly Val Thr His  
115 120 125  
Val Cys Asp Pro Ser Val Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys  
130 135 140  
Ile Ser Lys Ala Trp Val Cys Asp Gly Asp Asn Asp Cys Glu Asp Asn  
145 150 155 160  
Ser Asp Glu Glu Asn Cys Glu Ser Leu Ala Cys Arg Pro Pro Ser His  
165 170 175  
Pro Cys Ala Asn Asn Thr Ser Val Cys Leu Pro Pro Asp Lys Leu Cys  
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<212> PRT  
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35 40 45

<210> 46  
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<212> PRT  
<213> Homo sapiens

<400> 46

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 20 25 30  
 Glu Thr His Ala Asn Cys Thr Asn Gln Ala Thr Arg Pro Pro Gly Gly  
 35 40 45  
 Cys His Thr Asp Glu Phe Gln Cys Arg Leu Asp Gly Leu Cys Ile Pro  
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<210> 47  
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<400> 47  
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 Glu His Trp Thr Cys Asp Gly Asp Asn Asp Cys Gly Asp Tyr Ser Asp  
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 35 40 45  
 Cys His Thr Asp Glu Phe Gln Cys Arg Leu Asp Gly Leu Cys Ile Pro  
 50 55 60  
 Leu Arg Trp Arg Cys Asp Gly Asp Thr Asp Cys Met Asp Ser Ser Asp  
 65 70 75 80  
 Glu Lys Ser Cys Glu Gly Val Thr His Val Cys Asp Pro Ser Val Lys  
 85 90 95  
 Phe Gly Cys Lys Asp Ser Ala Arg Cys Ile Ser Lys Ala Trp Val Cys  
 100 105 110  
 Asp Gly Asp Asn Asp Cys Glu Asp Asn Ser Asp Glu Glu Asn Cys Glu  
 115 120 125  
 Ser Leu Ala Cys Arg Pro Pro Ser His Pro Cys Ala Asn Asn Thr Ser  
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 Val Cys Leu Pro Pro Asp Lys Leu Cys Asp Gly Asn Asp Asp Cys Gly  
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 Asp Gly Ser Asp Glu Gly Glu Leu Cys Asp  
 165 170

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 Gly Cys His Thr Asp Glu Phe Gln Cys Arg Leu Asp Gly Leu Cys Ile  
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<210> 49  
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<213> Homo sapiens

<400> 49

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Gly Cys His Thr Asp Glu Phe Gln Cys Arg Leu Asp Gly Leu Cys Ile
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 20                               25                               30
Asp Glu Lys Ser Cys Glu Gly Val Thr His Val Cys Asp Pro Ser Val
 35                               40                               45
Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys Ile Ser Lys Ala Trp Val
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Cys Asp Gly Asp Asn Asp Cys Glu Asp Asn Ser Asp Glu Glu Asn Cys
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<211> 123

<212> PRT

<213> Homo sapiens

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Asp Glu Lys Ser Cys Glu Gly Val Thr His Val Cys Asp Pro Ser Val
 35                               40                               45
Lys Phe Gly Cys Lys Asp Ser Ala Arg Cys Ile Ser Lys Ala Trp Val
 50                               55                               60
Cys Asp Gly Asp Asn Asp Cys Glu Asp Asn Ser Asp Glu Glu Asn Cys
 65                               70                               75                               80
Glu Ser Leu Ala Cys Arg Pro Pro Ser His Pro Cys Ala Asn Asn Thr
                               85                               90                               95
Ser Val Cys Leu Pro Pro Asp Lys Leu Cys Asp Gly Asn Asp Asp Cys
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Gly Asp Gly Ser Asp Glu Gly Glu Leu Cys Asp
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<210> 51

<211> 41

<212> PRT

<213> Homo sapiens

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Ile Ser Lys Ala Trp Val Cys Asp Gly Asp Asn Asp Cys Glu Asp Asn
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Ser Asp Glu Glu Asn Cys Glu Ser Leu
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<210> 52

<211> 81

<212> PRT

<213> Homo sapiens

<400> 52

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 20 25 30  
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 35 40 45  
 Pro Cys Ala Asn Asn Thr Ser Val Cys Leu Pro Pro Asp Lys Leu Cys  
 50 55 60  
 Asp Gly Asn Asp Asp Cys Gly Asp Gly Ser Asp Glu Gly Glu Leu Cys  
 65 70 75 80  
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<210> 53  
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 <213> Homo sapiens

<400> 53  
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 35 40

<210> 54  
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 <212> PRT  
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<400> 54  
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<210> 55  
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 <212> PRT  
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<400> 55  
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 1 5 10

<210> 56  
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<400> 56  
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<210> 57  
 <211> 11  
 <212> PRT  
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<400> 57  
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1 5 10

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US01/18041

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC(7) : C12N 5/00, 15/00; C12P 21/06; G01N 33/53; A61K 38/00  
 US CL : 485/325, 69.1, 69.3, 7.1, 7.2, 7.21, 7.23; 514/12;  
 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. : 485/325, 69.1, 69.3, 7.1, 7.2, 7.21, 7.23; 514/12;

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)  
 Medline, Biosis, Embase, Scisearch, WPIDS, UsPatfull  
 search terms: alpha2 macroglobulin receptor and heat shock protein, alpha 2 receptor ligand, antigen presentation

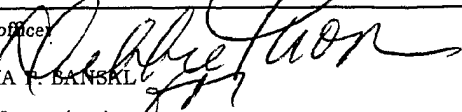
**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	BINDER et al. CD91: a receptor for heat shock protein gp96. Nature Immunol. August 2000. Vol. 1 No.2. pages 151-155.	1-96

Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 12 AUGUST 2001	Date of mailing of the international search report <b>26 SEP 2001</b>
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer  GEETHA P. BANSAI Telephone No. (703) 305-0196
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专利名称(译)	α ( 2 ) 巨球蛋白受体作为热休克蛋白受体及其用途		
公开(公告)号	<a href="#">EP1290140A4</a>	公开(公告)日	2004-06-09
申请号	EP2001941889	申请日	2001-06-04
[标]申请(专利权)人(译)	康涅狄格大学健康中心		
申请(专利权)人(译)	康涅狄格卫生中心大学		
当前申请(专利权)人(译)	康涅狄格卫生中心大学		
[标]发明人	SRIVASTAVA PRAMOD K		
发明人	SRIVASTAVA, PRAMOD, K.		
IPC分类号	G01N33/50 A61K35/12 A61K35/14 A61K38/00 A61K38/16 A61K38/17 A61K39/00 A61K39/395 A61K45/00 A61P31/00 A61P35/00 A61P37/02 A61P37/04 C07K14/705 C07K16/18 C07K16/28 C12N5/06 C12N5/10 C12N15/09 C12Q1/02 C12Q1/68 G01N33/15 G01N33/53 G01N30/02		
CPC分类号	A61K35/14 A61K38/00 A61K39/0011 A61K2039/5152 A61K2039/5156 A61P31/00 A61P35/00 A61P37/02 A61P37/04 C07K14/70596 C07K16/18 C07K16/2896 G01N33/92 G01N2333/705 G01N2500/02 A61K39/001176		
优先权	60/209095 2000-06-02 US 09/625137 2000-07-25 US 09/668724 2000-09-22 US 09/750972 2000-12-28 US		
其他公开文献	EP1290140A1		
外部链接	<a href="#">Espacenet</a>		

#### 摘要(译)

本发明涉及α ( 2 ) 巨球蛋白 ( “α2M” ) 受体作为热休克蛋白受体的用途，表达与HSP结合的α2M受体的细胞，以及结合α2M受体-HSP复合物的抗体和其他分子。。本发明还涉及筛选试验，以鉴定与α2M受体相互作用的化合物，并调节α2M受体与其配体 ( 如HSP ) 的相互作用，以及使用包含α2M-受体序列的组合物诊断和治疗免疫的方法。疾病，增殖性疾病和传染病。