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(54) **COMPOSITIONS AND METHOD FOR
TREATING CARDIOVASCULAR DISEASE**

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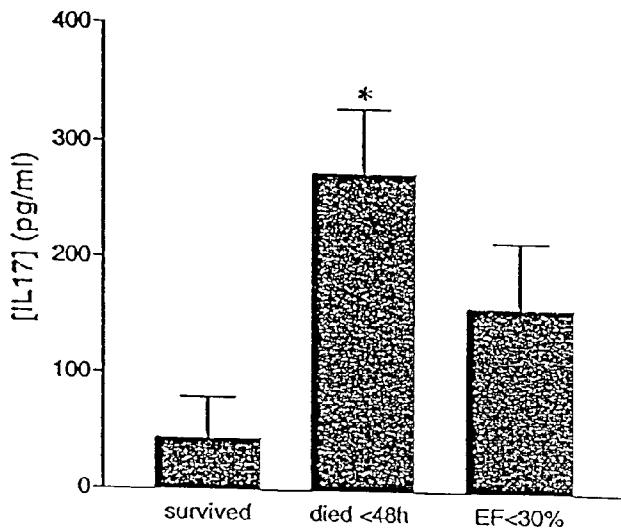
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ABSTRACT

The invention pertains to methods of treating cardiovascular disease by modulating inflammatory and immunoregulatory responses associated with such pathological conditions. Embodiments of the invention provide methods for the treatment of cardiovascular disease in a subject having cardiovascular disease comprising administering an effective amount of one or more IL-17 antagonists, IL-18 antagonists, 4-1BB antagonists, CD30 antagonists, OX40 antagonists and/or CD39 alone or in any combination. This abstract is provided for the sole purpose of enabling the reader to quickly ascertain the subject matter of the technical disclosure and is not intended to be used to interpret or limit the scope or meaning of the claims.

[IL17] in heart donor plasma

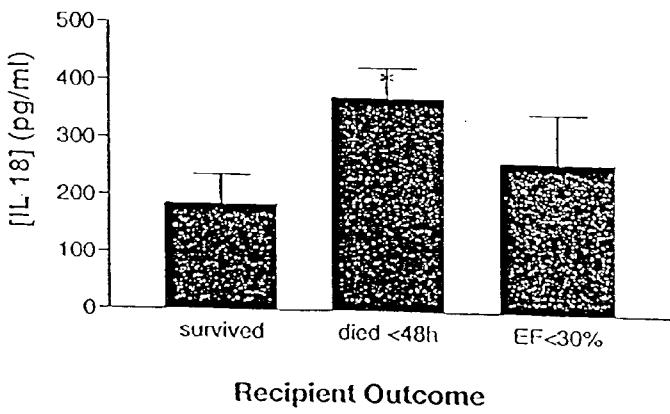
1-A



Recipients surviving
Recipients died
<48h
Unused Donors with
EF <30%

[IL 18] in heart donor plasma

1-B



Recipients surviving
Recipients died
<48h
Unused Donors with
EF >30%

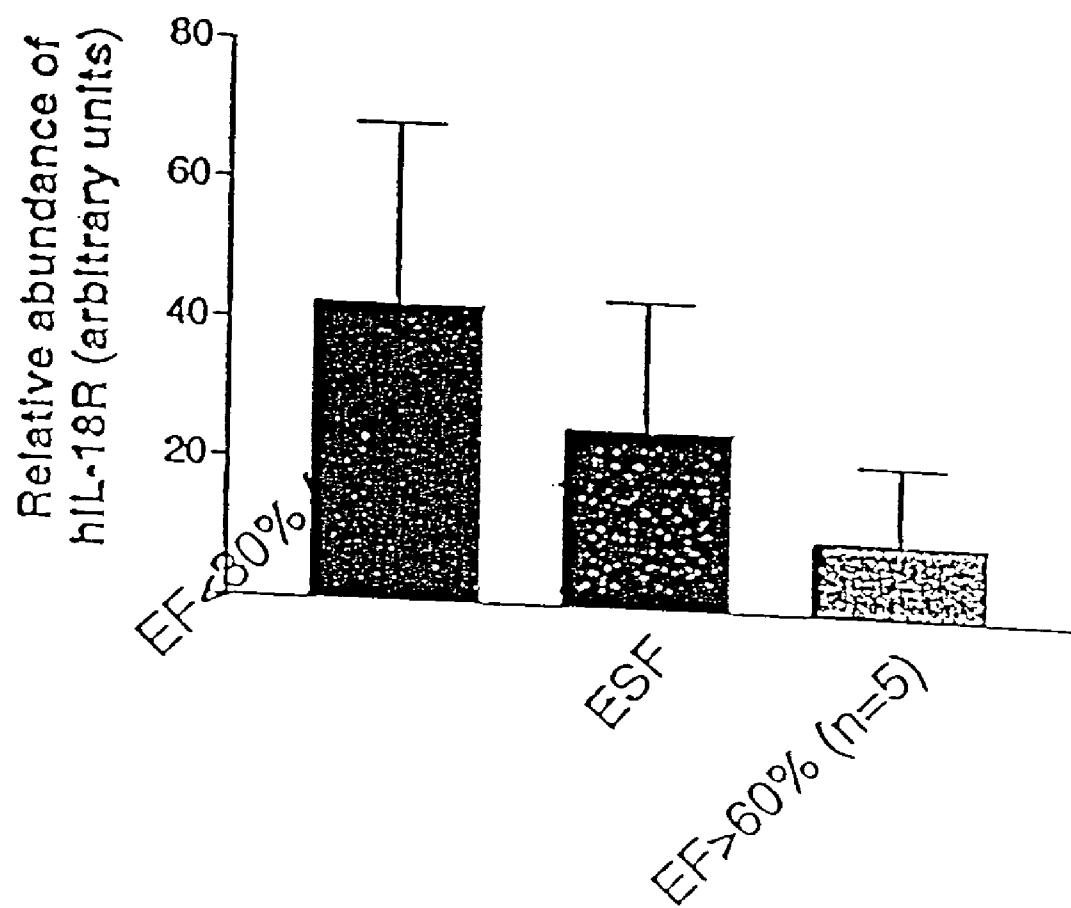
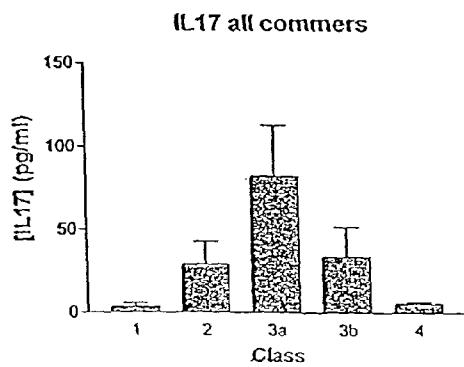
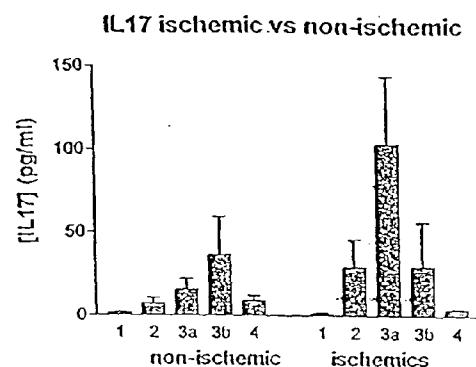


FIG. 2

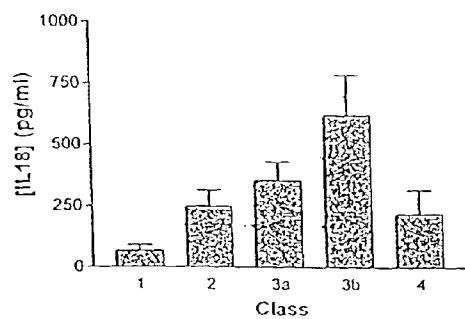
3-A



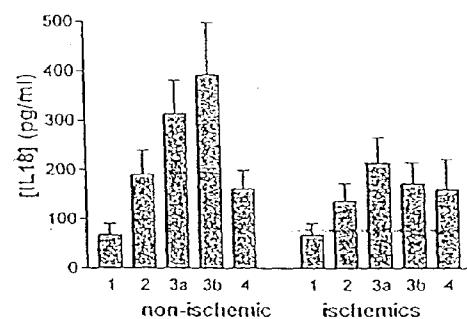
3-B



IL18 all commers



IL18



3-C

3-D

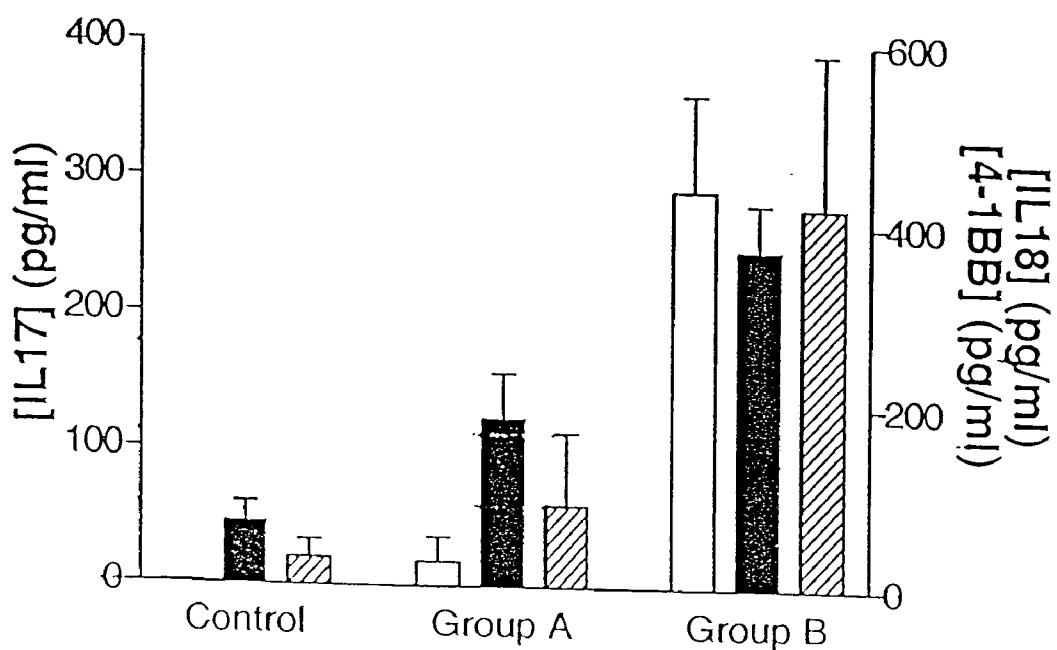


FIG. 4

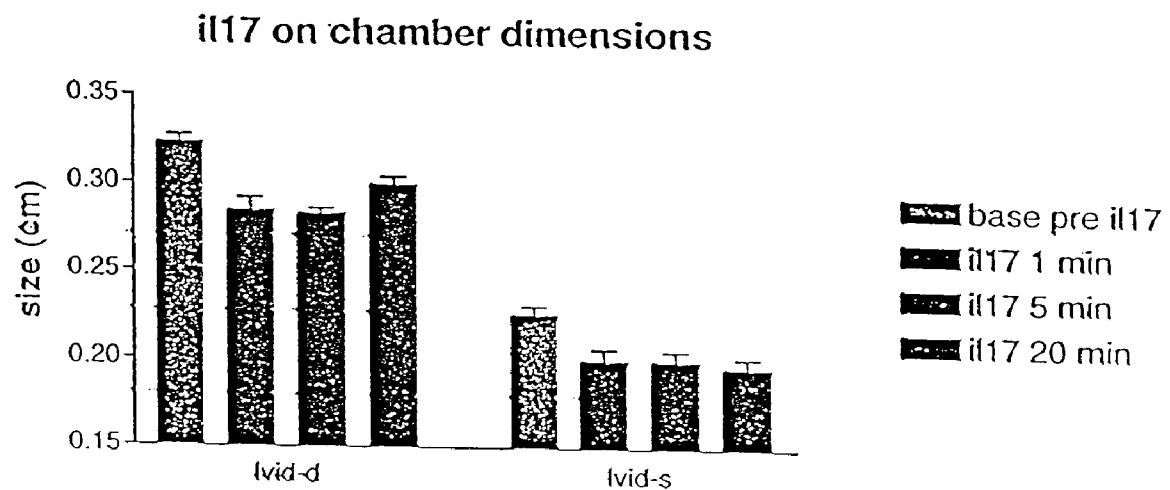
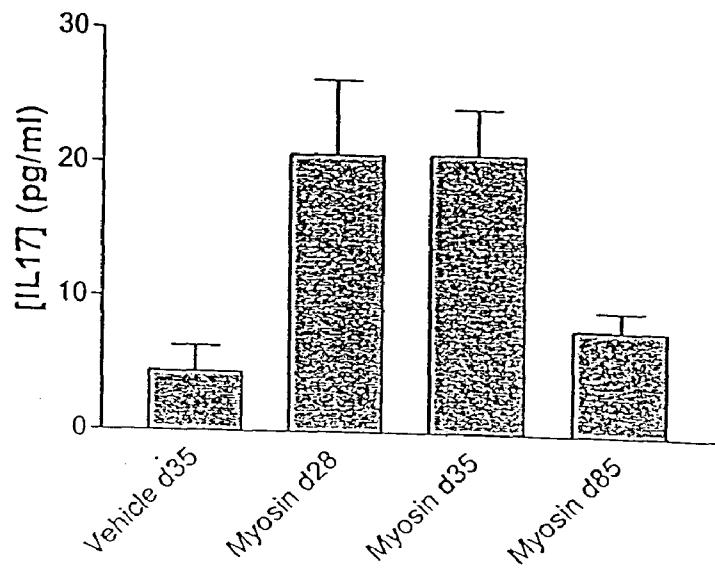


FIG. 5

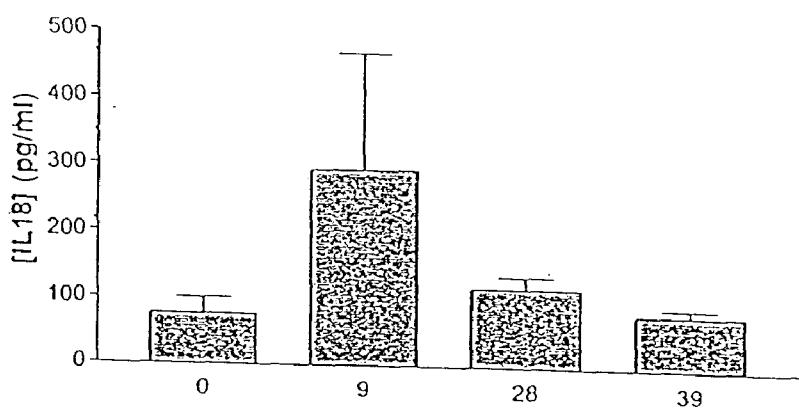
Plasma IL17 levels in IFN γ -/- mice immunized with Myosin peptide

6-A

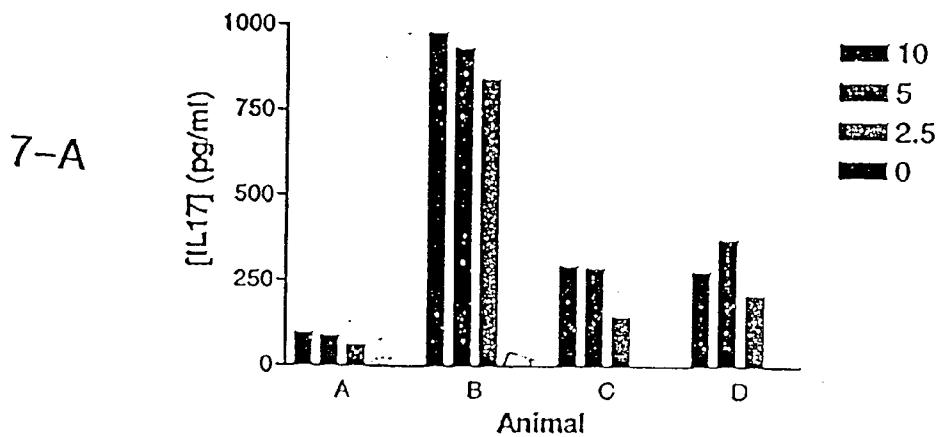


[IL18]

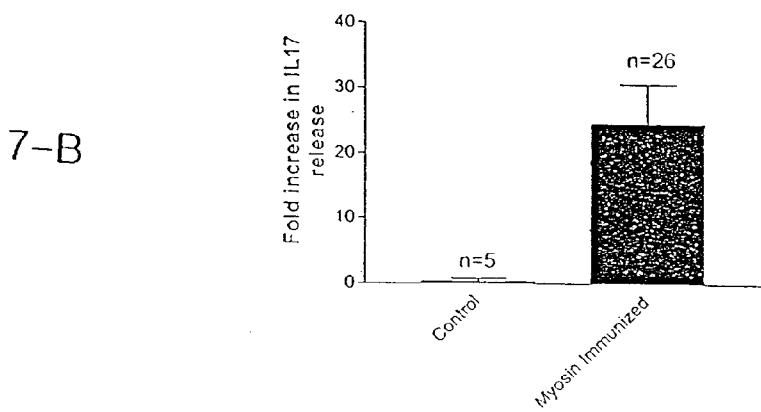
6-B



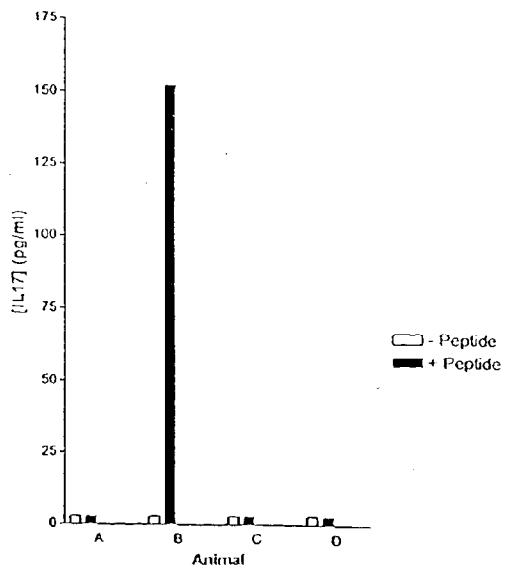
**IL17 production from T-cells
stimulated with anti-CD3.**



**Fold increase in IL17 release
myosin fed APC :T-cell cultures**



7-C



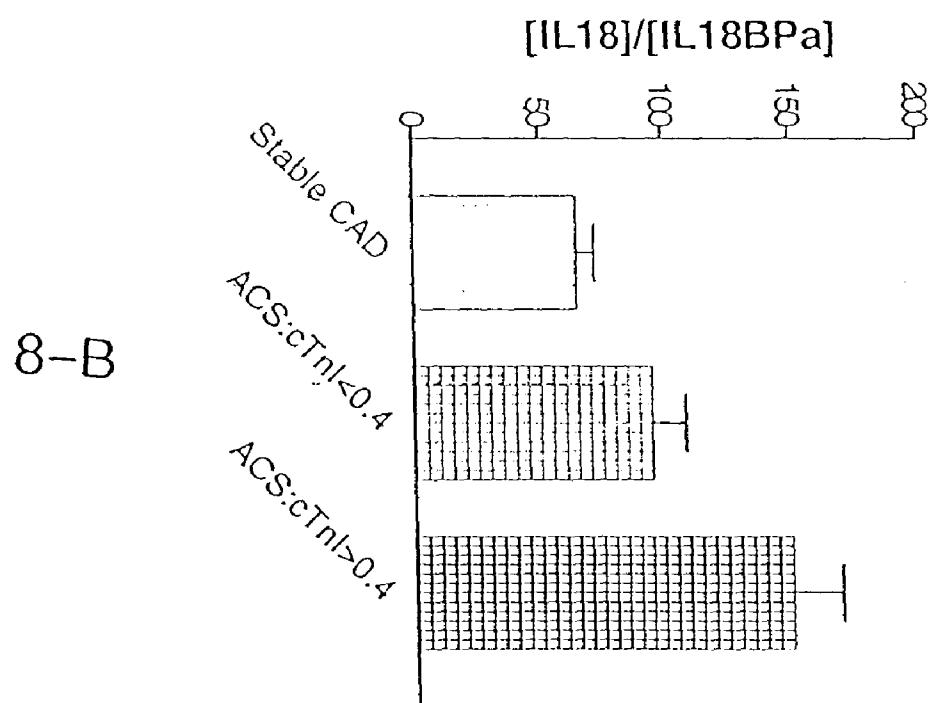
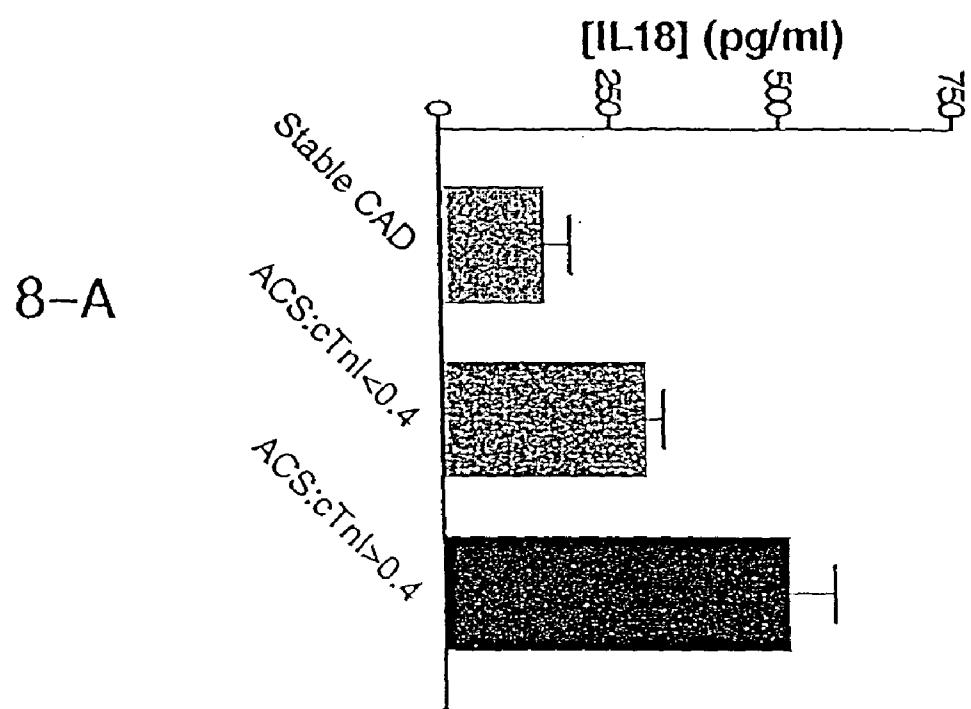


Figure 9

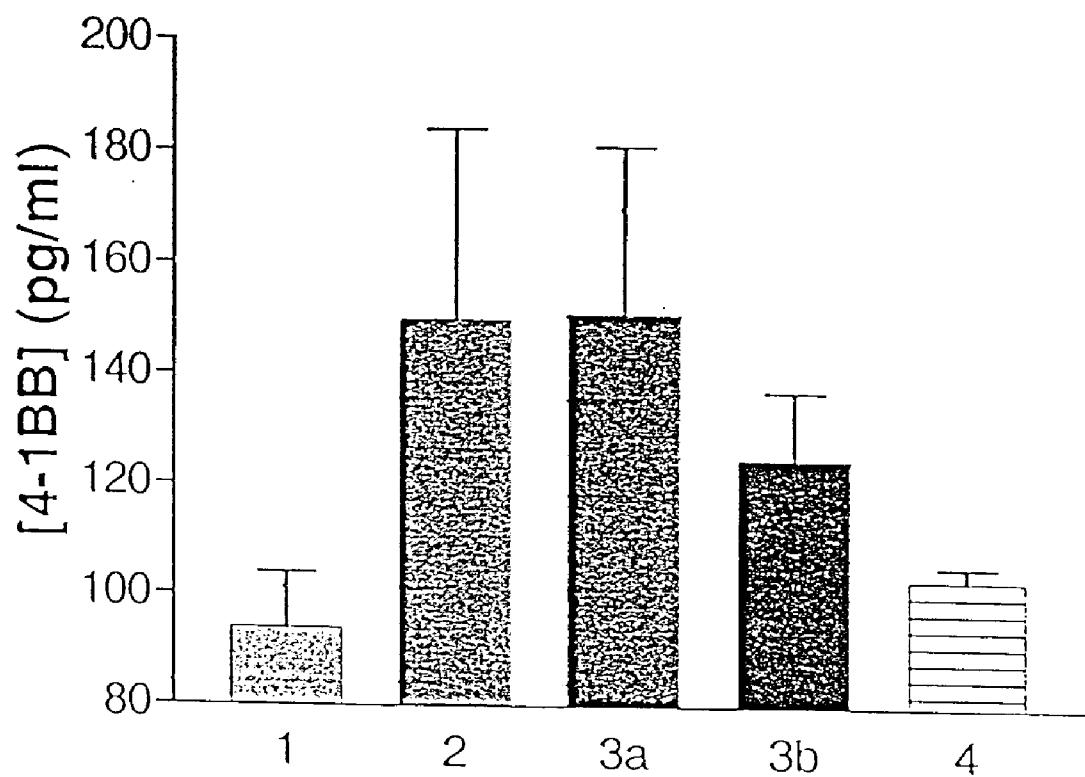


Figure 10.

Adriamycin Dose Response in 4-1BBL-mice

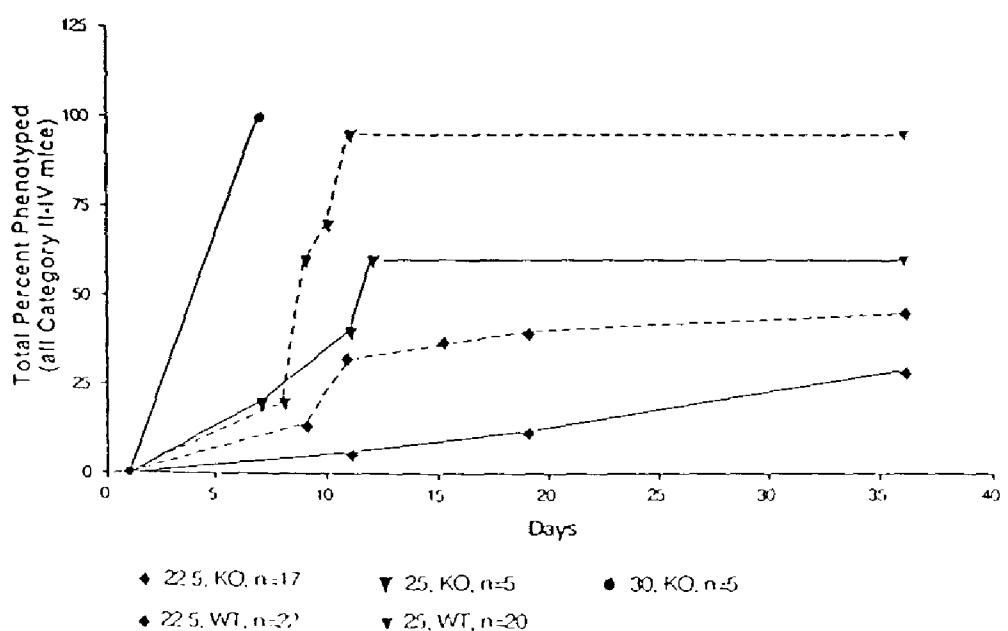
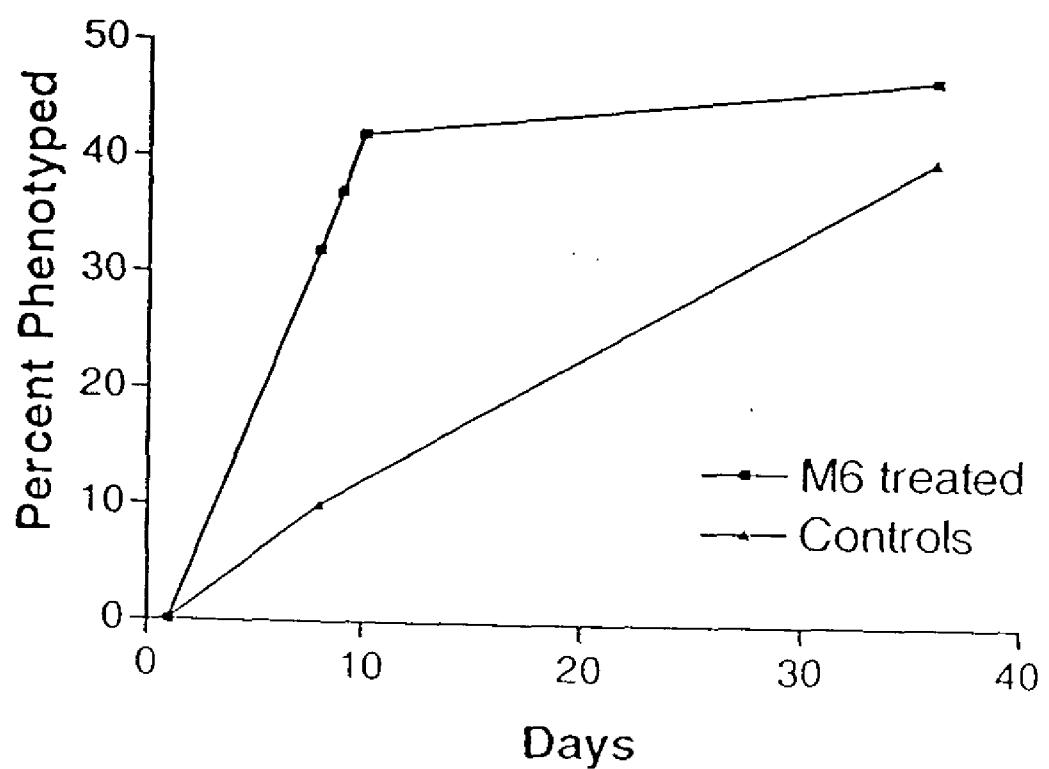


Figure 11

**COMPOSITIONS AND METHOD FOR TREATING
CARDIOVASCULAR DISEASE****CROSS REFERENCE TO RELATED
APPLICATIONS**

[0001] This application claims the benefit under 35 U.S.C. §119 of U.S. Provisional Application Serial No. 60/494,457, filed Aug. 12, 2003, and U.S. Provisional Application Serial No. 60/406,418, filed Aug. 28, 2002.

FIELD OF THE INVENTION

[0002] Embodiments of the invention pertain to compositions and methods for treating cardiovascular disease by modulating inflammatory and immunoregulatory responses associated with cardiovascular disease.

BACKGROUND

[0003] Cardiovascular disease encompasses a number of disorders that affect the muscle and/or blood vessels of the heart, peripheral blood vessels, muscles and various organs. It is established in the art that inflammatory and immunoregulatory processes are implicated in the pathogenesis of various forms of cardiovascular disease.

[0004] For example, inflammatory immune responses have been shown to contribute to the pathogenesis of atherosclerosis. Elevated levels of C-reactive protein (CRP) have been associated with up to an 8.6 fold increase in the relative risk of symptomatic atherosclerosis (Biasucci, L., et al., *Circulation* 1999, 99:855-860). Elevated levels of CRP also predict patients that are at elevated risk of myocardial infarction (MI) or stroke, and it has also been associated with poor prognosis in unstable angina (Vorchheimer, D., et al., *JAMA* 2001, 286:2154-2156). Binding C1q CRP activates the classical complement pathway and may lead to direct myocardial damage, coronary smooth muscle or endothelial cell death and subsequent atherosclerotic plaque rupture (Agrawal, A., et al., *J Immunol* 2001, 166:3998-4004). Furthermore, a recent study demonstrated that elevated levels of CRP are able to identify patients that die of sudden cardiac death 9 years prior to the event (Albert, C., et al., *Circulation* 2002, 105:2595-9). These studies also imply that long-term inflammatory exposure and elevated CRP levels may contribute to the progression of acute coronary syndromes (Buffon, A., et al., *NEJM* 2002, 347:55-7). Activation of inflammatory cells resident within an atherosclerotic plaque may elaborate enzymes capable of degrading extracellular matrix and lead to plaque rupture. Alternatively, these inflammatory cells may directly kill endothelial and smooth muscle cells. A number of studies have demonstrated that patients with unstable angina have peripheral T-cells that make enhanced levels of interferon gamma compared to patients with stable angina. Furthermore, there appears to be clonal expansion of a CD4+ CD28null T-cell population in these patients, which appear to be cytotoxic and can kill endothelial cells, an effect enhanced by CRP (Nakajima, T., et al., *Circulation* 2002, 105:570-5).

[0005] Cytokines are critical regulators of the T-helper 1 (Th1) and Th2 T-cell responses. The Th1 response results in pro-inflammatory cytokine release characterized by macrophage activation and, if unopposed, may result in tissue damage. The Th2 response results in a humoral immune

response, B-cell activation and an allergic reaction (Neurath, M., et al., *Nat Med* 2002, 8:567-73). A number of Th1 type cytokines including TNF, IL-6 and the chemokine MCP-1 are elevated in unstable angina (AJC 2001, 88(8A):10K-15K). Recently, IL-18 has been found to be an independent marker for an adverse outcome in patients diagnosed with acute coronary syndrome (Blankenberg, S., et al., *Circulation* 2002, 106:24-30). Elevated levels of IL-18 have also been found to correlate with ulcerated, symptomatic carotid artery lesions (Mallat, Z., et al., *Circulation* 2001, 104:1598-603). In a mouse model of atherosclerotic plaque development in ApoE deficient mice, IL-18 was shown to accelerate and enhance plaque formation, and IL-18 binding protein enhanced smooth muscle proliferation, which would promote plaque stability by increasing the thickness of the cap, and reduced the number of infiltrating macrophages and T-cells (Mallat, Z., et al., *Circ Res*. 2001, 89:E41-5). Studies such as these establish a sound basis for implicating inflammatory and immunoregulatory responses in cardiovascular disease.

[0006] There is an unmet need in the art for treating cardiovascular disease by targeting the immunopathology of the disease. Embodiments of the present invention address such needs by providing compositions and methods for treating cardiovascular disease by modulating the inflammatory and immunoregulatory responses associated with cardiovascular disease.

SUMMARY OF THE INVENTION

[0007] Embodiments of the present invention provide compositions and methods for the treatment of cardiovascular disease in a subject having cardiovascular disease, comprising administering an effective amount of one or more antagonists, such as IL-17 antagonists that inhibit the binding of IL-17 to the IL-17 receptor, as well as antagonists that prevent or diminish the activation of the IL-17 receptor; IL-18 antagonists that inhibit the binding of IL-18 to the IL-18 receptor, as well as antagonists that prevent or diminish the activation of the IL-18 receptor; 4-1BB antagonists that inhibit the binding of 4-1BB ligand to 4-1BB, as well as antagonists that prevent or diminish the activation of 4-1BB ligand or 4-1BB; CD30 antagonists that inhibit the binding of CD30 ligand to CD30, as well as antagonists that prevent or diminish the activation of the CD30; OX40 antagonists that inhibit the binding of OX40 ligand to OX40, as well as antagonists that prevent or diminish the activation of OX40; and/or CD39 alone or in any combination.

[0008] Additional embodiments are described in detail below.

BRIEF DESCRIPTION OF THE FIGURES

[0009] FIGS. 1A and 1B depict IL-17 and IL-18 levels, respectively, in heart donor plasma in relation to three distinct groups: surviving donor recipients, donor recipients that died in less than 48 hrs. and unused donors with an ejection fraction (EF) of less than 30%.

[0010] FIG. 2 shows relative abundance of IL-18 Receptor in patients having an ejection fraction (EF) of less than 30%, patients in end-stage failure (ESF) and patients having an ejection fraction of greater than 60% (i.e., normal myocardium).

[0011] FIGS. 3A through 3D show comparative levels of IL-17 and IL-18 plasma levels among patients in various stages of cardiomyopathy. FIGS. 3B and 3D further dissect the patient population into non-ischemic and ischemic groups.

[0012] FIG. 4 illustrates the plasma levels of IL-17 (open bars) and IL-18 (closed bars) and 4-1BB (hatched bars) of control subjects or donors with favorable recipient outcome (group A) or adverse recipient outcome (group B) post-transplantation.

[0013] FIG. 5 illustrates the effect of administering IL-17 on heart chamber dimensions over time.

[0014] FIGS. 6A and 6B depict IL-17 and IL-18 plasma levels in IFN- $\gamma^{-/-}$ mice immunized with cardiac myosin peptide that induces an inflammatory myocarditis and cardiomyopathy.

[0015] FIGS. 7A through 7C: FIG. 7A shows IL-17 levels in mice from the experimental autoimmune myocarditis (EAM) model, wherein mice having histologically demonstrated cardiopathology (animal B) had higher expression levels of IL-17 over negative controls (animals C and D). FIG. 7B shows that IL-17 release from T-cells obtained from mice with EAM mice was approximately 25 fold higher compared to control mice. T-cells were isolated from animals immunized with cardiac myosin and exposed to antigen presenting cells fed myosin over antigen presenting cells not exposed to myosin. FIG. 7C illustrates that T-cells from an animal immunized with cardiac myosin and having histological evidence of cardiopathology (animal B) released high levels of IL-17 in response to antigen-specific stimulation by peptide-pulsed antigen presenting cells.

[0016] FIGS. 8A and 8B show IL-18 levels and the ratio of IL-18 to IL-18 Binding Protein, respectively, in patients stratified into three patient groups: those having stable coronary artery disease (CAD), acute coronary syndrome (ACS) with cardiac troponin I (cTnI) plasma levels less than 0.4 ng/ml or acute coronary syndrome (ACS) with cardiac troponin I (cTnI) plasma levels greater than 0.4 ng/ml.

[0017] FIG. 9 illustrates that 4-1BB plasma levels were significantly elevated in patients with heart failure compared to normal subjects thereby suggesting activation of this system in human heart failure.

[0018] FIG. 10 represents a dose-response of Adriamycin® in wild type and 4-1BBL-/- knock out mice. At 22.5 mg/kg and 25 mg/kg, the percentage of Adriamycin®-induced cardiac dysfunction was decreased in 4-1BBL-/- At high dosage (30 mg/kg), no difference was observed between wild type and 4-1BBL-/- group.

[0019] FIG. 11 is a graph showing 4-1BB activating antibody (M6) administered 3, 6, 9 days after Adriamycin® treatment. The onset of dysfunction was accelerated by M6 antibody, but the final penetrance was similar to control group.

DETAILED DESCRIPTION OF THE INVENTION

[0020] Embodiments of the invention provide compositions and methods for treating cardiovascular disease in a subject having cardiovascular disease comprising administering an effective amount of one or more IL-17 antagonists,

IL-18 antagonists, 4-1BB antagonists, CD30 antagonists, OX40 antagonists and/or CD39, alone or in any combination.

[0021] Cardiovascular disease, as defined herein, encompasses diseases and disorders of the muscle and/or blood vessels of the heart, diseases and disorders of the vascular system, and/or diseases and disorders of organs and anatomical systems caused by the diseased condition of the heart and/or vasculature. Examples include, but are not limited to: inflammation of the heart and/or vasculature such as myocarditis, chronic autoimmune myocarditis, bacterial and viral myocarditis, as well as infective endocarditis; heart failure; congestive heart failure; chronic heart failure; cachexia of heart failure; cardiomyopathy, including non-ischemic (dilated cardiomyopathy; idiopathic dilated cardiomyopathy; cardiogenic shock, heart failure secondary to extracorporeal circulatory support ("post-pump syndrome"), heart failure following ischemia/reperfusion injury, brain death associated heart failure (as described in Owen et al., 1999 (Circulation. 1999 May 18;99(19):2565-70)); hypertrophic cardiomyopathy; restrictive cardiomyopathy; non-ischemic systemic hypertension; valvular disease; arrhythmicogenic right ventricular cardiomyopathy) and ischemic (atherogenesis; atherosclerosis; arteriosclerosis; peripheral vascular disease; coronary artery disease; infarctions, including stroke, transient ischemic attacks and myocardial infarctions). Additional disease states encompassed by the definition of cardiovascular disease include: aneurysms; arteritis; angina; embolism; platelet-associated ischemic disorders; ischemia/reperfusion injury; restenosis; mitral and/or tricuspid regurgitation; mitral stenosis; silent myocardial ischemia; Raynaud's phenomena; thrombosis; deep venous thrombosis; pulmonary embolism; thrombotic microangiopathies including thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS), essential thrombocythemia, disseminated intravascular coagulation (DIC), and thrombosis and coagulopathies associated with exposure to a foreign or injured tissue surface; thrombophlebitis; vasculitis, including Kawasaki's vasculitis; Takayasu's arteritis; veno-occlusive disease, giant cell arteritis, Wegener's granulomatosis; Schoenlein-Henoch purpura, as well as cardiovascular disease arising from periodontal infections by one or more oral pathogens, such as bacteria. The examples of cardiovascular disease provided above are merely illustrative and provided to aid those of skill in the art to appreciate the scope of cardiovascular disease that may be treated using the compositions and methods described herein. Of course, other cardiovascular disease conditions may exist that can be treated using the inventive compositions and methods. Additional examples of cardiovascular disease and disorders associated with cardiovascular disease, as well as complications arising from the treatment of cardiovascular disease, are provided in the section below pertaining to therapeutic indications.

[0022] An "antagonist," as defined herein, is a molecule that partially or completely blocks the binding of two cognates thereby inhibiting the downstream biological effects of the cognates' interaction. For example, an antagonist may block the binding of a ligand to its receptor, which in turn reduces and/or prevents intracellular signalling via activating that receptor, which in turn reduces or prevents the downstream biological effects of activating that receptor, such as but not limited to, cell activation, proliferation,

differentiation, cytokine release, up-regulation of genes, cell-surface expression of proteins, and the like.

[0023] Activating or activation of a receptor is defined herein as the engagement of one or more intracellular signaling pathway(s) and the transduction of intracellular signaling (i.e., signal transduction) in response to a molecule binding to a membrane-bound receptor, such as but not limited to, a receptor:ligand interaction.

[0024] "Signal transduction," as used herein, is the relaying of a signal by conversion from one physical or chemical form to another. In cell biology, the process by which a cell converts an extracellular signal into a response.

[0025] Antagonists presented herein comprise soluble receptor molecules, ligands and/or binding proteins, including IL-17, IL-17 receptor (IL-17R), IL-18, IL-18 receptor (IL-18R), IL-18 binding protein (IL-18BP), CD30, CD30 ligand (CD30-L), 4-1BB, 4-1BB ligand (4-1BB-L), OX40, OX40 ligand (OX40-L) and CD39. Antagonists presented herein further comprise antibodies, fusion proteins and peptibodies directed against one or more of the following: IL-17, IL-17R, IL-18, IL-18R, CD30, CD30-L, 4-1BB, 4-1BB-L, OX40 and/or OX40-L. Antagonists presented herein further comprise small molecules, such as peptidomimetics and mimotopes, and the like, that antagonize the interaction between IL-17 and IL-17R, IL-18 and IL-18R, 4-1BB and 4-1BB-L, CD30 and CD30-L and/or OX40 and OX40-L. Additional antagonists comprise antisense oligonucleotides that specifically target and hybridize to the mRNA of IL-17, IL-17R, IL-18, IL-18R, CD30, CD30-L, 4-1BB, 4-1BB-L, OX40 and/or OX40-L thereby preventing gene translation of their respective proteins. Further embodiments comprise gene silencing by RNA-interference molecules tailored to silence expression of IL-17, IL-17R, IL-18, IL-18R, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40 and/or OX40-L. More specific definitions and examples of particular antagonists are provided in the sections below.

[0026] A "peptibody" refers to molecules comprising an Fc domain and at least one peptide. Such peptibodies may be multimers or dimers or fragments thereof, and they may be derivatized. Peptibodies are described in greater detail in WO 99/25044 and WO 00/24782, which are incorporated herein by reference in their entirety. The peptide may be from the amino acid sequence of IL-17, IL-17 receptor (IL-17R), IL-18, IL-18 receptor (IL-18R), IL-18 binding protein (IL-18BP), CD30, CD30 ligand (CD30-L), 4-1BB, 4-1BB ligand (4-1BB-L), OX40, OX40 ligand (OX40-L) and/or CD39.

[0027] A "peptide," as used herein refers to molecules of 1 to 40 amino acids. Alternative embodiments comprise molecules of 5 to 20 amino acids. Exemplary peptides may comprise portions of the extracellular domain of naturally occurring molecules or comprise randomized sequences of IL-17, IL-17 receptor (IL-17R), IL-18, IL-18 receptor (IL-18R), IL-18 binding protein (IL-18BP), CD30, CD30 ligand (CD30-L), 4-1BB, 4-1BB ligand (4-1BB-L), OX40, OX40 ligand (OX40-L) and/or CD39.

[0028] The term "randomized" as used to refer to peptide sequences refers to fully random sequences (e.g., selected by phage display methods or RNA-peptide screening) and sequences in which one or more residues of a naturally occurring molecule is replaced by an amino acid residue not

appearing in that position in the naturally occurring molecule. Exemplary methods for identifying peptide sequences include phage display, *E. coli* display, ribosome display, RNA-peptide screening, chemical screening, and the like.

[0029] The term "Fc domain" encompasses native Fc and Fc variant molecules and sequences as defined below. As with Fc variants and native Fc's, the term "Fc domain" includes molecules in monomeric or multimeric form, whether digested from whole antibody or produced by other means.

[0030] The term "native Fc" refers to molecule or sequence comprising the sequence of a non-antigen-binding fragment resulting from digestion of whole antibody, whether in monomeric or multimeric form. The original immunoglobulin source of the native Fc is preferably of human origin and may be any of the immunoglobulins, although IgG1 and IgG2 are preferred. Native Fc's are made up of monomeric polypeptides that may be linked into dimeric or multimeric forms by covalent (i.e., disulfide bonds) and non-covalent association. The number of intermolecular disulfide bonds between monomeric subunits of native Fc molecules ranges from 1 to 4 depending on class (e.g., IgG, IgA, IgE) or subclass (e.g., IgG1, IgG2, IgG3, IgA1, IgA2). One example of a native Fc is a disulfide-bonded dimer resulting from papain digestion of an IgG (see Ellison et al. (1982), *Nucleic Acids Res.* 10: 4071-9). The term "native Fc" as used herein is generic to the monomeric, dimeric, and multimeric forms.

[0031] The term "Fc variant" refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the salvage receptor, FcRn. International applications WO 97/34631 (published 25 Sep. 1997) and WO 96/32478 describe exemplary Fc variants, as well as interaction with the salvage receptor, and are hereby incorporated by reference in their entirety. Thus, the term "Fc variant" comprises a molecule or sequence that is humanized from a non-human native Fc. Furthermore, a native Fc comprises sites that may be removed because they provide structural features or biological activity that are not required for the fusion molecules of the present invention. Thus, the term "Fc variant" comprises a molecule or sequence that lacks one or more native Fc sites or residues that affect or are involved in (1) disulfide bond formation, (2) incompatibility with a selected host cell (3) N-terminal heterogeneity upon expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or (7) antibody-dependent cellular cytotoxicity (ADCC). Fc variants are described in further detail hereinafter.

[0032] A "peptidomimetic" is a peptide analog that displays more favorable pharmacological properties than their prototype native peptides, such as a) metabolic stability, b) good bioavailability, c) high receptor affinity and receptor selectivity, and d) minimal side effects. Designing peptidomimetics and methods of producing the same are known in the art (see for example, U.S. Pat. Nos. 6,407,059 and 6,420,118). Peptidomimetics may be derived from the binding site of the extracellular domain of IL-17, IL-17 receptor (IL-17R), IL-18, IL-18 receptor (IL-18R), IL-18 binding protein (IL-18BP), CD30, CD30 ligand (CD30-L), 4-1BB, 4-1BB ligand (4-1BB-L), OX40, OX40 ligand (OX40-L) and/or CD39. In alternative embodiments, a peptidomimetic

comprises non-peptide compounds having the same three-dimensional structure as peptides derived from IL-17, IL-17 receptor (IL-17R), IL-18, IL-18 receptor (IL-18R), IL-18 binding protein (IL-18BP), CD30, CD30 ligand (CD30-L), 4-1BB, 4-1BB ligand (4-1BB-L), OX40, OX40 ligand (OX40-L) and/or CD39, or compounds in which part of a peptide from the molecules listed above is replaced by a non-peptide moiety having the same three-dimensional structure.

[0033] A "mimotope" is defined herein as peptide sequences that mimic binding sites on proteins (see, Partidos, CD, et al., *Combinatorial Chem & High Throughput Screening*, 2002 5:15-27). A mimotope may have the capacity to mimic a conformationally-dependent binding site of a protein. The sequences of these mimotopes do not identify a continuous linear native sequence or necessarily occur in a naturally-occurring protein. Mimotopes and methods of production are taught in U.S. Pat. No. 5,877,155 and U.S. Pat. No. 5,998,577, which are incorporated by reference in their entirieties.

[0034] The term "acidic residue" refers to amino acid residues in D- or L-form having sidechains comprising acidic groups. Exemplary acidic residues include D and E.

[0035] The term "amide residue" refers to amino acids in D- or L-form having sidechains comprising amide derivatives of acidic groups. Exemplary residues include N and Q.

[0036] The term "aromatic residue" refers to amino acid residues in D- or L-form having sidechains comprising aromatic groups. Exemplary aromatic residues include F, Y, and W.

[0037] The term "basic residue" refers to amino acid residues in D- or L-form having sidechains comprising basic groups. Exemplary basic residues include H, K, and R.

[0038] The term "hydrophilic residue" refers to amino acid residues in D- or L-form having sidechains comprising polar groups. Exemplary hydrophilic residues include C, S, T, N, and Q.

[0039] The term "nonfunctional residue" refers to amino acid residues in D- or L-form having sidechains that lack acidic, basic, or aromatic groups. Exemplary nonfunctional amino acid residues include M, G, A, V, I, L and norleucine (Nle).

[0040] The term "neutral hydrophobic residue" refers to amino acid residues in D- or L-form having sidechains that lack basic, acidic, or polar groups. Exemplary neutral hydrophobic amino acid residues include A, V, L, I, P, W, M, and F.

[0041] The term "polar hydrophobic residue" refers to amino acid residues in D- or L-form having sidechains comprising polar groups. Exemplary polar hydrophobic amino acid residues include T, G, S, Y, C, Q, and N.

[0042] The term "hydrophobic residue" refers to amino acid residues in D- or L-form having sidechains that lack basic or acidic groups. Exemplary hydrophobic amino acid residues include A, V, L, I, P, W, M, F, T, G, S, Y, C, Q, and N.

[0043] The term "subject" as used herein, refers to mammals. For example, mammals contemplated by the present invention include humans; primates; pets of all sorts, such as

dogs, cats, etc.; domesticated animals, such as, sheep, cattle, goats, pigs, horses and the like; common laboratory animals, such as mice, rats, rabbits, guinea pigs, etc.; as well as captive animals, such as in a zoo or free wild animals. Throughout the specification, the term host is used interchangeably with subject.

[0044] As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an immunization" includes a plurality of such immunizations and reference to "the cell" includes reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs unless clearly indicated otherwise.

[0045] It is understood that the various embodiments of this invention are not limited to the particular methodology, protocols, cell lines, animal species or genera, constructs, and reagents described, as such may vary. It is also understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

I. IL-17, IL-18, 4-1BB, CD30 and OX40 Antagonists

[0046] A. IL-17 Antagonists

[0047] Embodiments of the present invention provide compositions and methods for the treatment of cardiovascular disease comprising IL-17 antagonists. Studies presented herein demonstrate that IL-17 and IL-18 are elevated in human patients having various forms and severity of cardiovascular disease. These studies demonstrate that circulating levels of IL-17 and/or IL-18 correlate with severity of cardiovascular disease. Furthermore, plasma levels of IL-17 and/or IL-18 are elevated in a cardiac myosin-induced myocarditis model and correlate with disease severity. Therefore, IL-17 and/or IL-18 are implicated in cardiovascular disease and provide a rational basis for treating cardiovascular disease by administering IL-17 and/or IL-18 antagonists, alone or in combination. In addition, IL-17 and/or IL-18 are prognostic indicators of cardiovascular disease and disease severity. IL-17 and/or IL-18 are also prognostic indicators of donor adequacy and post-transplant outcome. Therefore, further embodiments of the invention include assays for measuring IL-17 and/or IL-18 levels in subjects being screened for cardiovascular disease, cardiovascular disease severity, donor adequacy and post-transplant outcome.

[0048] An IL-17 antagonist is defined herein as an entity that is capable of reducing the effective amount of endogenous IL-17 in a subject, by either partially or completely blocking the interaction of IL-17 and the IL-17 receptor and thereby inhibiting IL-17-mediated signaling via membrane-bound IL-17 receptor, as well as partially or completely inhibiting the subsequent biological effects of activating the IL-17 receptor. An IL-17 antagonist may bind to IL-17 or to the IL-17 receptor.

[0049] Such IL-17 antagonists include, but are not limited to: soluble forms of IL-17 receptor; antibodies directed

against IL-17 that specifically bind IL-17 and partially or completely inhibit binding of IL-17 to IL-17 receptor; antibodies, fusion proteins and/or peptibodies directed against IL-17 receptor that specifically bind IL-17 receptor and inhibit binding of IL-17 without themselves activating the IL-17 receptor; molecules that bind IL-17 or IL-17 receptor and inhibit the interaction thereof, such as IL-17 and/or IL-17 receptor peptidomimetics and/or mimotopes. As used herein, when reference is made to making IL-17 antagonists based on IL-17 or IL-17 receptor, it is understood that the terms IL-17 and IL-17 receptor also encompass fragments, variants, muteins, derivatives and fusion proteins thereof, as described in detail below.

[0050] Biological activity of IL-17 and IL-17R is defined herein as comprising binding of IL-17 to the IL-17R and activation of the IL-17R; proinflammatory effects; increased production of cytokines and chemokines, such as but not limited to, IL-6, IL-8, G-CSF, GM-CSF, MCP-1, Gro α , PGE2, as well as induction of costimulatory molecule ICAM. The IL-17:IL-17R interaction also has the biological activities of recruiting monocytes and neutrophils, up-regulation of iNOS, NO and COX-2; activation of all three subgroups of MAPKs (the p44 and p42 extracellular signal-regulated kinases ERK1 and ERK2), NF κ B, stress-induced Jun NH₂-terminal kinases (JNK1 and JNK2) and p38. Of course, it is understood that intermediate pathways that culminate in such biological activities are also included in the definition of biological activity for IL-17 and IL-17R.

[0051] IL-17 antagonists may comprise or be developed from IL-17 receptor polypeptide and/or polynucleotide sequences, as well as fragments, variants, muteins, derivatives and fusion proteins thereof. The isolation, cloning, preparation and characterization of human IL-17 receptor (referred to interchangeably as IL-17R or hIL-17R) are described in U.S. Pat. No. 5,869,286 and U.S. Pat. No. 6,072,033, which are incorporated herein by reference in their entirety. The full-length cDNA sequence for human IL-17R is provided in SEQ ID NO:3 and the corresponding amino acid sequence is provided in SEQ ID NO:4.

[0052] The human IL-17 receptor has an N-terminal signal peptide with a predicted cleavage site between amino acid 27 and 28. The signal peptide is followed by a 293 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 525 amino acid cytoplasmic tail. Soluble polypeptides are polypeptides that are capable of being secreted from the cells in which they are expressed. A secreted soluble polypeptide may be identified (and distinguished from its non-soluble membrane-bound counterparts) by separating intact cells which express the desired polypeptide from the culture medium, e.g., by centrifugation, and assaying the medium (supernatant) for the presence of the desired polypeptide. The presence of the desired polypeptide in the medium indicates that the polypeptide was secreted from the cells and thus is a soluble form of the polypeptide. The use of soluble forms of IL-17 receptor is advantageous for many applications. Purification of the polypeptides from recombinant host cells is facilitated, since the soluble polypeptides are secreted from the cells. Moreover, soluble polypeptides are generally more suitable than membrane-bound forms for parenteral administration and for many enzymatic procedures. Soluble forms of IL-17R that are useful in the methods of treating cardiovascular disease include the extracellular domain (residues 1-320 of

SEQ ID NO:4 or residues 28-320 which excludes the signal peptide) or a fragment of the extracellular domain that has the properties of antagonizing or preventing binding of IL-17 to endogenous IL-17R. Soluble IL-17R also includes those polypeptides which include part of the transmembrane region, provided that the soluble IL-17R is capable of being secreted from a cell, and preferably retains the capacity to bind IL-17 and effectuate its biological effects.

[0053] Other forms of the IL-17R that are useful in the present invention include muteins and variants (also referred to as analogs), such as naturally occurring variants, that are substantially homologous to the native IL-17R of SEQ ID NO:4 and as described in U.S. Pat. No. 6,072,033 that retain biological activity of IL-17R.

[0054] The invention further encompasses IL-17 antagonists derived from IL-17R polynucleotide sequences. Embodiments of the invention include full length nucleic acid molecules encoding soluble IL-17R as well as isolated fragments and oligonucleotides derived from the nucleotide sequence of SEQ ID NO:3. Such nucleic acid sequences may include nucleotides 178-1494 of SEQ ID NO:3 or a fragment thereof, and DNA and/or RNA sequences that hybridize to the coding region of the nucleotide sequence of SEQ ID NO:3, or its complement, under conditions of moderate stringency, and which encode polypeptides or fragments thereof of the invention.

[0055] In other embodiments, IL-17 antagonists may comprise or be developed from IL-17 polynucleotide and/or polypeptide sequences. The full-length cDNA sequence for human IL-17 is provided in SEQ ID NO: 1 and the corresponding amino acid sequence is provided in SEQ ID NO: 2. Commercially available recombinant human IL-17 is also available, for example, from R&D Systems, Minneapolis, MN. IL-17 polypeptides, as well as biologically active fragments or derivatives thereof, may be used to generate antibodies that specifically bind to IL-17 and have the capacity of partially or completely blocking IL-17 binding to the IL-17 receptor.

[0056] In further embodiments, IL-17 antagonists are small molecules and polypeptide mimetics, such as but not limited to, peptidomimetics, peptibodies and/or mimotopes developed from the polypeptide sequence of IL-17 (SEQ ID NO:2) and/or IL-17R (SEQ ID NO:4). Polypeptide mimetics are peptide-containing molecules which mimic elements of protein secondary structure. Polypeptide mimetics, such as peptidomimetics and mimotopes, may be developed through techniques known in the art, such as combinatorial peptide libraries.

[0057] An IL-17 polypeptide mimetic based on the amino acid sequence of IL-17 will bind to IL-17R without activating the IL-17R and sterically hinder binding of endogenous IL-17. Conversely, an IL-17R polypeptide mimetic based on the amino acid sequence of IL-17R will bind to IL-17 and sterically hinder IL-17 from binding to endogenous IL-17R. IL-17 peptide mimetics may be used to antagonize IL-17 in a subject and thereby reduce the proinflammatory effects of IL-17. As such, IL-17 polypeptide mimetics may be used to treat inflammatory and/or immunoregulatory processes associated with cardiovascular disease.

[0058] Other forms of the IL-17 that are useful in the present invention include muteins and variants (also referred

to as analogs), such as naturally occurring variants, that are substantially homologous to the native IL-17 of SEQ ID NO:2 that retain biological activity of IL-17. For example, IL-17 homologues B, C, D, E and F. This invention additionally provides for the use of IL-17 antagonists in the manufacture of a medicament for the treatment of cardiovascular disease. This invention further provides for the use of polynucleotides encoding IL-17 antagonists in the manufacture of a medicament for the treatment of cardiovascular disease.

[0059] B. IL-18 Antagonists

[0060] Embodiments of the present invention provide compositions and methods for the treatment of cardiovascular disease comprising IL-18 antagonists. An IL-18 antagonist is defined herein as an entity that is capable of reducing the effective amount of endogenous IL-18, by either partially or completely blocking the interaction of IL-18 and the IL-18R and thereby inhibiting IL-18-mediated signaling via membrane-bound IL-18R, as well as partially or completely inhibiting the subsequent biological effects of activating the IL-18 receptor. An IL-18 antagonist may bind to IL-18 or to the IL-18R. Antagonists derived from the IL-18R and IL-18 Binding Protein (e.g. soluble forms that bind IL-18) compete for IL-18 with IL-18R on the cell surface, thus inhibiting IL-18 from binding to cells, thereby preventing IL-18 from manifesting its biological activities.

[0061] IL-18: IL-18R biological activity, is defined herein as including, but is not limited to, binding of IL-18 to the IL-18R and activation of the IL-18R; regulation of innate and acquired immune responses; proinflammatory effects; induction of T-lymphocyte helper cell type 1 responses (Th1); enhance cell-mediated cytotoxicity; IFN- γ induction; enhanced production of GM-CSF and IL-2; potentiation of anti-CD3 induced T-cell proliferation; increased Fas-mediated killing by natural killer cells (NK cells) and CD4+ Th1 cells; increased apoptotic death via the Fas-FasL pathway; up-regulation of FasL expression; induction of T-lymphocyte helper cell type 2 responses (Th2) in T-cells and NK cells; stimulation of basophils and mast cells to produce Th2 cytokines and histamine; induction of IgE production

[0062] Such IL-18 antagonists include, but are not limited to: soluble forms of IL-18R; IL-18 Binding Protein; antibodies directed against IL-18 that specifically bind IL-18 and partially or completely inhibit binding of IL-18 to IL-18R; antibodies, fusion proteins and/or peptobodies directed against IL-18R that specifically bind IL-18R and inhibit receptor binding of IL-18 without themselves transducing a signal via IL-18R; small molecules that bind IL-18 or IL-18R that inhibit the interaction thereof, such as IL-18 and/or IL-18R peptidomimetics and/or mimotopes. As used herein, when reference is made to making IL-18 antagonists based on IL-18, IL-18 Binding Protein or IL-18 receptor, it is understood that the terms IL-18, IL-18 Binding Protein and IL-18 receptor also encompass fragments, variants, muteins, derivatives and fusion proteins thereof, as described in detail below.

[0063] The isolation, cloning, preparation and characterization of human IL-18 receptor (referred to interchangeably as IL-18R or huIL-18R) are described in U.S. Pat. No. 6,087,116 and U.S. patent application Ser. No. 09/621,502 (PCT Publication WO 99/37772), which are incorporated herein by reference in their entirety. The IL-18 receptor is a

heterodimeric protein containing an IL-18 binding subunit termed IL-1Rrp1, and an accessory subunit termed AcPL. Although the IL-1Rrp1 subunit alone will bind IL-18, its affinity for IL-18 is increased dramatically when present in a heterodimeric complex with the AcPL subunit.

[0064] The IL-1Rrp1 polynucleotide sequence and corresponding amino acid sequence that it encodes are provided as SEQ ID NO:5 and SEQ ID NO:6, respectively. The soluble extracellular portion of the IL-1Rrp1 subunit that binds IL-18 is represented by amino acids 20 to 329 of SEQ ID NO:6; cleavage of the signal sequence occurs just after amino acid residue 19 of SEQ ID NO:6. However, fragments as small as amino acid residues 20 to 123 and amino acid residues 20 to 226 of SEQ ID NO:6 have been reported to bind IL-18 and can also be used. The IL-1Rrp1 polypeptide is also described in U.S. Pat. No. 5,776,731, incorporated in its entirety by reference herein.

[0065] The AcPL polynucleotide sequence and the amino acid sequence that it encodes are provided herein as SEQ ID NO:7 and SEQ ID NO:8, respectively. The mature extracellular domain of AcPL consists of amino acids 15 to 356 of SEQ ID NO:8; cleavage of the signal sequence occurs just after amino acid residue 14 of SEQ ID NO:8. The AcPL polypeptide, and soluble extracellular fragments thereof, are also described in in U.S. patent application Ser. No. 09/621,502 (PCT Publication WO 99/37772), incorporated herein by reference in its entirety.

[0066] One embodiment of a soluble form of an IL-18 receptor for use in the methods of the present invention comprises amino acids 1-329 sequence of SEQ ID NO:6; alternative embodiments of a soluble form of an IL-18 receptor comprises amino acids 20-329 after cleavage of the signal sequence of SEQ ID NO:6. A further embodiment of a soluble form of IL-18 receptor is a heterodimeric receptor that includes at least amino acid residues 20-123, 20-226 or 20-329 of SEQ ID NO:6 (the IL-1Rrp1 subunit), and at least amino acids 15-340 of SEQ ID NO:8 (the AcPL subunit), in a covalent or non-covalent association.

[0067] Additional IL-18 antagonists comprise the IL-18 Binding Protein. PCT Publication WO 99/09063 describes the IL-18 binding protein, including useful soluble fragments thereof. One embodiment of a human IL-18 Binding Protein is the "a" isoform having the polynucleotide sequence of SEQ ID NO:9 and the corresponding amino acid sequence of SEQ ID NO:10. Of course, other IL-18 Binding Protein isoforms that are antagonistic to IL-18 binding to IL-18R may be used, such as the b, c and d isoforms. The polynucleotide and amino acid sequences for the b, c and d isoforms are known in the art and readily available (see for example, Kim, S.-H., et al., *PNAS* 97:3 1190-1195 (2000)). A particularly useful form of the IL-18 binding protein is a fusion with an Fc domain of an antibody. The amino acid sequence of an example of such a fusion protein, termed IL-18BP-Fc herein, is presented in SEQ ID NO:11. This 422 amino acid protein, when expressed in a mammalian cell, will be secreted; the mature secreted form of the protein contains amino acid residues 29-422. Of these residues, amino acid residues 29-192 represent the IL-18 binding protein portion of the molecule, and amino acid residues 193-422 represent the Fc portion of the molecule. The Fc region facilitates purification and dimerization of the fusion polypeptide.

[0068] IL-18 antagonists may also comprise or be developed from IL-18 polynucleotide and/or polypeptide sequences. Human IL-18 has been recombinantly produced from a cloned cDNA, as described in U.S. Pat. No. 5,891,663 and cloned genomic DNA, as disclosed in U.S. Pat. No. 6,060,283, which are incorporated by reference in their entirety. The full-length cDNA sequence is provided in SEQ ID NO:12 with the corresponding amino acid sequence in SEQ ID NO:13. The amino acid sequence for ICE-processed human IL-18 provided in SEQ ID NO:14. Commercially available recombinant human IL-18 is available, for example, from R&D Systems, Minneapolis, MN. IL-18 polypeptides as well as biologically active fragments or derivatives thereof may be used to generate antibodies that specifically bind to IL-18 and have the capacity of partially or completely blocking IL-18 binding to the IL-18 receptor.

[0069] In one embodiment, IL-18 antagonists are polypeptide mimetics, such as, but not limited to peptidomimetics, peptibodies and/or mimotopes developed from the polypeptide sequence of IL-18 (SEQ ID NO:13 and/or 14). Polypeptide mimetics may be developed through techniques known in the art, such as combinatorial peptide libraries. Polypeptide mimetics are peptide-containing molecules which mimic elements of protein secondary structure. An IL-18 polypeptide mimetic based on the amino acid sequence of IL-18 will bind to IL-18 receptor without activating the IL-18 receptor and sterically hinder binding of endogenous IL-18. IL-18 peptide mimetics may be used to antagonize IL-18 in a subject and thereby reduce the proinflammatory effects of IL-18. As such, IL-18 polypeptide mimetics may be used to treat inflammatory and/or immunoregulatory processes associated with cardiovascular disease.

[0070] Other embodiments of IL-18, IL-18 receptor and IL-18 Binding Protein that may be used as IL-18 antagonists include muteins and variants (as described in greater detail below), such as naturally occurring variants, that are substantially homologous to the native IL-18 of SEQ ID NO:13 and/or 14, IL-18 receptor of SEQ ID NOs:6 and 8, and IL-18 Binding Protein of SEQ ID NO:10 that retain biological activity. Biological activity, in this instance, is the capacity to bind its cognate partner.

[0071] This invention additionally provides for the use of IL-18 antagonists in the manufacture of a medicament for the treatment of cardiovascular disease. This invention further provides for the use of polynucleotides encoding IL-18 antagonists in the manufacture of a medicament for the treatment of cardiovascular disease.

[0072] C. 4-1BB Antagonists

[0073] Further embodiments of the present invention provide compositions and methods for the treatment of cardiovascular disease comprising 4-1BB antagonists.

[0074] Examples 7 and 8 describe studies demonstrating a role for the 4-1BB/4-1BBL immune co-stimulatory pathway in Adriamycin®-induced cardiomyopathy, as well as demonstrating a novel cardiac expression pattern of 4-1BB and implicating apoptosis as a mechanism of co-stimulatory contribution to Adriamycin®-induced cardiomyopathy. More specifically, 4-1BBL deficient mice and 4-1BBL decoy receptor-treated mice conferred partial resistance to adriamycin induced cardiac damage, whereas 4-1BB activating antibody accelerated onset of damage, implying the contri-

bution of 4-1BB to Adriamycin® effects in heart. Apoptosis, measured by TUNEL, sub-G1 DNA and activated caspase-3, was increased in Adriamycin®-treated wild type myocardium, but reduced in 4-1BBL-/- mice. Phosphorylation of Akt was selectively suppressed by Adriamycin®, but maintained by loss of 4-1BBL, indicating the modulation of apoptosis by co-stimulatory pathway in heart is possibly through Akt, but not Jnk and p38 signaling. The consistency of decreased index of apoptosis and the improved cardiac function in 4-1BBL-/- suggests apoptosis play a pivotal role in Adriamycin®-induced cardiac deficiency.

[0075] A single retroorbital (RO) injection of adriamycin (22.5 mg/kg) leads to progressive cardiac dysfunction without evidence of inflammatory infiltration. In this model of non-inflammatory, drug-induced cardiomyopathy, 4-1BBL-/- mice have substantially improved cardiac function by echocardiography. Furthermore, m4-1BB Fc (a soluble decoy receptor for 4-1BBL) reduced ADR cardiac dysfunction, while an agonistic antibody to 4-1BB (M6) accelerated and exacerbated cardiac dysfunction. While no inflammatory infiltrate is observed in this Adr-cardiomyopathy, we found expression of 4-1BB induced on 1-5% of cardiac interstitial cells within 2 days after Adr. Cardiac apoptosis, measured by TUNEL and sub-G1 DNA, is increased 3 days after ADR(45 mg/kg), concomitant with the increased expression of 4-1BB on interstitial cells. Chronic ongoing apoptosis, determined 5 weeks after Adr challenge when cardiac dysfunction is maximal in wild type but largely absent in 4-1BBL-/- mice, was lower in 4-1BBL-/- mice (1.5-fold vs baseline), compared to WT mice (4 fold). In a separate study, caspase 3 activation, determined by Western blot, was increased at 48 to 72 hrs post-ADR (45 mg/kg). In contrast, ADR did not induce caspase 3 cleavage in 4-1BBL-/- myocardium. Determined by western blot, adriamycin reduced phosphorylation of Akt in wild type but not 4-1BBL-/- hearts. Phosphorylation of JNK and p38 was not impacted by Adr. In summary, 4-1BB/4-1BBL immune co-stimulatory pathway contributes to ADR-induced cardiomyopathy, possibly, through modulation of Akt signaling to regulate apoptosis in the heart.

[0076] A 4-1BB antagonist is defined herein as an entity that is capable of reducing the effective amount of available endogenous 4-1BB and/or 4-1BB ligand (4-1BB-L), by either partially or completely blocking the interaction of 4-1BB and 4-1BB-L and thereby inhibiting 4-1BB-mediated signaling via 4-1BB-L and 4-1BB-L-mediated signaling via 4-1BB, as well as the subsequent biological effects of activating 4-1BB and/or 4-1BB-L. In other words, because the 4-1BB:4-1BB-L interaction exhibits bi-directional signalling, a 4-1BB antagonist may bind either 4-1BB or 4-1BB-L so long as the antagonist does not itself activate 4-1BB or 4-1BB-L. Such 4-1BB antagonists include, but are not limited to: soluble forms of 4-1BB; antibodies, fusion proteins and/or peptibodies directed against 4-1BB that specifically bind 4-1BB and partially or completely inhibit binding of 4-1BB to 4-1BB-L; antibodies, fusion proteins and/or peptibodies directed against 4-1BB that specifically bind 4-1BB and inhibit binding of 4-1BB-L without themselves transducing a signal via 4-1BB; molecules that bind 4-1BB or 4-1BB-L and inhibit the interaction thereof, such as 4-1BB and/or 4-1BB-L small molecules, peptidomimetics and/or mimotopes, and/or polypeptides comprising all or portions of 4-1BB or 4-1BB-L or modified variants thereof,

including genetically-modified muteins, multimeric forms and sustained-release formulations thereof.

[0077] 4-1BB:4-1BB-L biological activity, is defined herein as including, but is not limited to, binding of 4-1BB-L to 4-1BB and activation of one or both of 4-1BB and 4-1BB-L; costimulatory activity on T lymphocytes; activation and differentiation of CD4+ and CD8+ cells; signal transduction through TRAF pathways (TRAF1, TRAF2 and TRAF3) and activation of NF κ B and AP-1; inhibition of activation-induced cell death; facilitation of B-cell proliferation and monocyte activation; up-regulation of cytokines including, but not limited to, IL-6, IL-8 and TNF- α ; up-regulation of adhesion molecules, such as ICAM; down-regulation of Fc γ RIII; production of M-CSF in monocytes; monocyte proliferation; and inhibition of T-cell proliferation induced by anti-CD3 antibodies.

[0078] 4-1BB antagonists may comprise or be developed from 4-1BB-L polypeptide and polynucleotide sequences. The isolation, cloning, preparation and characterization of human 4-1BB-L (referred to interchangeably as hu4-1BB-L) is described in U.S. Pat. No. 5,674,704, which is incorporated herein by reference in its entirety. 4-1BB-L refers to a genus of mammalian polypeptides that are capable of binding 4-1BB. 4-1BB-L is a type II extracellular membrane polypeptide with an intracellular (cytoplasmic) domain at the N-terminus of the polypeptide (amino acids 1-25 of SEQ ID NO:15), followed by a transmembrane region polypeptide (amino acids 26-48 of SEQ ID NO:15), and an extracellular (receptor-binding) domain at the C-terminus of the polypeptide polypeptide (amino acids 49-254 of SEQ ID NO:15). Soluble 4-1BB-L polypeptides may be derived from the extracellular domain, as described below. The full-length cDNA sequence for human 4-1BB-L is provided in SEQ ID NO:14 and the corresponding amino acid sequence is provided in SEQ ID NO:15. The human 4-1BB-L protein comprises a cytoplasmic domain (amino acids 1-25), a transmembrane region (amino acids 26-48), and an extracellular domain (amino acids 49-254 of SEQ ID NO:15).

[0079] In addition, 4-1BB antagonists may comprise or be developed from 4-1BB polypeptide and polynucleotide sequences. The polynucleotide sequence of a human 4-1BB cDNA and the amino acid sequence encoded thereby are presented in SEQ ID NO:17 and SEQ ID NO:18, respectively. The human 4-1BB protein comprises an N-terminal signal sequence (amino acids -23 to -1 of SEQ ID NO:18), an extracellular domain comprising amino acids 1-163, a transmembrane region comprising amino acids 164-190, and a cytoplasmic domain comprising amino acids 191-232.

[0080] Soluble forms of 4-1BB-L and 4-1BB proteins are provided herein. Soluble 4-1BB-L or 4-1BB polypeptides comprise all or part of the extracellular domain but lack the transmembrane region that would cause retention of the polypeptide on a cell membrane. Since the 4-1BB-L protein lacks a signal peptide, a heterologous signal peptide may be fused to the N-terminus of soluble 4-1BB-L polypeptides to promote secretion thereof. The signal peptide is cleaved from the protein upon secretion from the host cell. Soluble 4-1BB-L polypeptides include fragments that retain the ability to bind 4-1BB, such as truncated polypeptides of the extracellular domain, and soluble 4-1BB polypeptides include fragments that retain the ability to bind 4-1BB-L,

such as truncated polypeptides of the extracellular domain 4-1BB. In alternative embodiments, the soluble proteins may include part of the transmembrane region or part of the cytoplasmic domain, provided that the protein is capable of being secreted rather than retained on the cell surface. Examples of soluble polypeptides include those comprising the entire extracellular domain. Specific examples include, but are not limited to a soluble human 4-1BB-L polypeptide comprising amino acids 49-254 of SEQ ID NO:16 and a soluble human 4-1BB polypeptide comprising amino acids 1-163 of SEQ ID NO:18.

[0081] In one embodiment, 4-1BB antagonists are polypeptide mimetics, such as but not limited to, peptidomimetics, peptibodies and/or mimotopes developed from the polypeptide sequence of 4-1BB-L (SEQ ID NO:16) and 4-1BB (SEQ ID NO:18). Polypeptide mimetics may be developed through techniques known in the art, such as combinatorial peptide libraries. Polypeptide mimetics are peptide-containing molecules which mimic elements of protein secondary structure. A 4-1BB polypeptide mimetic based on the amino acid sequence of 4-1BB will bind to 4-1BB-L without activating 4-1BB-L and sterically hinder binding of endogenous 4-1BB. Similarly, a 4-1BB-L polypeptide mimetic based on the amino acid sequence of 4-1BB-L will bind to 4-1BB without activating 4-1BB and sterically hinder binding of endogenous 4-1BB-L. 4-1BB and 4-1BB-L peptide mimetics may be used to antagonize their respective cognates in a subject and thereby reduce the proinflammatory effects of the 4-1BB/4-1BB-L interaction. As such, 4-1BB antagonists in the form of polypeptide mimetics may be used to treat inflammatory and/or immunoregulatory processes associated with cardiovascular disease.

[0082] Other embodiments of 4-1BB and 4-1BB-L that may be used as 4-1BB antagonists include muteins and variants (as described in greater detail below), such as naturally occurring variants, that are substantially homologous to the native 4-1BB-L (SEQ ID NO:16) and 4-1BB (SEQ ID NO:18) polypeptide sequences that retain biological activity. Biological activity, in this instance, is the capacity to bind its cognate partner.

[0083] This invention additionally provides for the use of 4-1BB antagonists in the manufacture of a medicament for the treatment of cardiovascular disease. This invention further provides for the use of polynucleotides encoding 4-1BB antagonists in the manufacture of a medicament for the treatment of cardiovascular disease.

[0084] D. CD30 Antagonists

[0085] Further embodiments of the present invention provide compositions and methods for the treatment of cardiovascular disease comprising one or more CD30 antagonists. A CD30 antagonist is defined herein as an entity that is capable of reducing the effective amount of endogenous CD30 ligand (CD30-L), by either partially or completely blocking the interaction of CD30-L and CD30 and thereby inhibiting CD30-mediated signaling via membrane-bound CD30, as well as partially or completely inhibiting the subsequent biological activity of activating CD30. A CD30 antagonist may bind to either CD30-L or CD30.

[0086] The biological activity of CD30: CD30-L includes, but is not limited to, binding of CD30-L to CD30 and

activation of CD30; intracellular activation of NF- κ B, cytokine release and/or proliferation of CD30+ cells; proliferation of T-cells in the presence of an anti-CD3 co-stimulus.

[0087] Such CD30 antagonists include, but are not limited to: soluble forms of CD30-L and CD30; fragments of CD30-L that bind CD30 and inhibit binding of CD30-L without activating membrane-bound CD30; fragments of CD30 that bind CD30-L and inhibit binding of CD30-L to CD30; antibodies, fusion proteins and/or peptibodies directed against CD30-L that specifically bind CD30-L and partially or completely inhibit binding of CD30-L to CD30; antibodies, fusion proteins and/or peptibodies directed against CD30 that specifically bind CD30 and inhibit binding of CD30-L without themselves activating the CD30; small molecules that bind CD30-L or CD30 and inhibit the interaction thereof, such as CD30-L and/or CD30 peptidomimetics and/or mimotopes. As used herein, when reference is made to CD30 antagonists based on CD30-L or CD30, it is understood that the terms CD30-L and CD30 also encompass fragments, variants, muteins, derivatives and fusion proteins thereof, as described in detail below.

[0088] CD30 antagonists may comprise or be developed from CD30-L polynucleotide and polypeptide sequences. The isolation, cloning, preparation and characterization of human CD30-L is described in U.S. Pat. No. 5,480,981, which is incorporated herein by reference in its entirety. As mentioned above, embodiments of the present invention include anti-CD30-L antibodies as CD30 antagonists. Examples of antibodies that are directed against CD30-L that may be used to treat cardiovascular disease are described in U.S. Pat. No. 5,677,430, which is incorporated by reference in its entirety.

[0089] The term "CD30-L" as used herein refers to a genus of polypeptides which are capable of binding CD30. As used herein, the term "CD30-L" includes membrane-bound proteins (comprising a cytoplasmic domain, a transmembrane region, and an extracellular domain) as well as truncated proteins that retain the CD30-binding property. Such truncated proteins include, for example, soluble CD30-L comprising only the extracellular (receptor binding) domain. CD30-L is expressed on monocytes/macrophages, granulocytes, a subset of B cells and on activated but not resting T cells. By binding with cell-surface CD30, CD30-L can induce murine B cell differentiation and can induce the proliferation of activated T cells in the presence of an anti-CD3 co-stimulus (see, for example, Smith et al., *Cell* 73:1349-1360 (1993)). Moreover, CD30-L exhibits "reverse signaling," that is, the cell surface CD30-L that is expressed on neutrophils and peripheral blood T cells can be activated by cross-linking to stimulate metabolic activities in those cells (Wiley et al., *J Immunol* 157: 3235-39 (1996)).

[0090] CD30-L proteins of the present invention include, but are not limited to, human CD30-L comprising amino acids 1-215 of SEQ ID NO:20 or 1-234 of SEQ ID NO:22; and proteins that comprise N-terminal, C-terminal, or internal truncations of the foregoing sequences, but retain the desired biological activity. Examples include human CD30-L proteins comprising amino acids y to 234 of SEQ ID NO:22 wherein y is 1-19 (i.e., the N-terminal amino acid is any one of amino acids 1-19 of SEQ ID NO:22, and amino acid 234 is the C-terminal amino acid. Such proteins, truncated at the N-terminus, are capable of binding CD30.

[0091] Alternative embodiments provide soluble CD30-L polypeptides. Soluble CD30-L polypeptides comprise all or part of the extracellular domain of a native CD30-L but lack the transmembrane region that would cause retention of the polypeptide on a cell membrane. Since the CD30-L protein lacks a signal peptide, a heterologous signal peptide may be fused to the N-terminus of a soluble CD30-L protein to promote secretion thereof. The signal peptide is cleaved from the CD30-L protein upon secretion from the host cell. The soluble CD30-L polypeptides retain the ability to bind the CD30 receptor. Soluble CD30-L may also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble CD30-L protein is capable of being secreted.

[0092] Examples of soluble CD30-L polypeptides include those comprising the entire extracellular domain of a native CD30-L protein or a fragment of said extracellular domain that is capable of binding CD30. One such soluble CD30-L polypeptides comprise amino acids z to 215 (Asp) of the human CD30-L sequence of SEQ ID NO:20, wherein z is 44, 45, 46, or 47. In other words, the N-terminal amino acid of the soluble human CD30-L is selected from the amino acids in positions 44-47 of SEQ ID NO:20. DNA sequences encoding such soluble human CD30-L polypeptides include, but are not limited to, DNA sequences comprising a nucleotide sequence selected from the group consisting of nucleotides 130-645, 133-645, 136-645, and 139-645 of SEQ ID NO:19. Such sequences encode polypeptides comprising amino acids 44-215, 45-215, 46-215, and 47-215, respectively, of SEQ ID NO:20. Production of one such soluble human CD30-L protein, in the form of a fusion protein comprising amino acids 47-215 of SEQ ID NO:20 and an antibody Fc polypeptide, is illustrated in Example 11 of U.S. Pat. No. 5,480,981.

[0093] CD30 antagonists may comprise or be developed from CD30 polypeptide and/or polynucleotide sequences. Cloning and expression of a gene encoding CD30 has been reported and CD30 has been characterized as a transmembrane protein that possesses substantial homology to the nerve growth factor receptor superfamily (Durkop et al., *Cell* 1992, 68:421). The CD30 polynucleotide sequence reported in Durkop et al. supra is presented in SEQ ID NO:23, and the amino acid sequence encoded thereby is presented in SEQ ID NO:24. The extracellular portion of human CD30 corresponds to amino acids 1-390, or if the signal peptide is removed, to amino acids 19-390 of SEQ ID NO:24. The transmembrane region comprises amino acids 391-407 of SEQ ID NO:24. The phrase "soluble CD30" (sCD30) refers to soluble molecules that comprise all or part of the extracellular domain of the CD30 protein, and that retain the capacity to bind specifically with CD30-L. The polynucleotide and polypeptide sequences, as well as a description of how to make a CD30-Fc fusion protein, which may serve as a CD30 antagonist for the treatment of cardiovascular disease, is described in detail in U.S. Pat. No. 5,480,981, which is incorporated herein by reference in its entirety.

[0094] In further embodiments, CD30 antagonists are polypeptide mimetics, such as, but not limited to peptidomimetics, peptibodies and/or mimotopes developed from the polypeptide sequence of CD30-L (SEQ ID NOs:20 and/or 22) and/or CD30 (SEQ ID NO:24). Polypeptide mimetics may be developed through techniques known in the art, such

as combinatorial peptide libraries. Polypeptide mimetics are peptide-containing molecules which mimic elements of protein secondary structure. A CD30-L polypeptide mimetic based on the amino acid sequence of CD30-L will bind to CD30 without activating CD30 and sterically hinder binding of endogenous CD30-L. A CD30 receptor polypeptide mimetic based on the amino acid sequence of CD30 receptor will bind to CD30-L and sterically hinder binding of endogenous CD30-L to CD30. CD30-L and CD30 peptide mimetics can be used to antagonize CD30-L binding to CD30 in a subject and thereby reduce the proinflammatory effects of CD30-L. As such, CD30 antagonists in the form of polypeptide mimetics may be used to treat inflammatory and/or immunoregulatory processes associated with cardiovascular disease.

[0095] Other forms of CD30-L and CD30 that are useful in the present invention include muteins and variants (also referred to as analogs), such as naturally occurring variants, that are substantially homologous to the native CD30-L (SEQ ID NOs:20 and/or 22) or CD30 (SEQ ID NO:24) polypeptide sequences and as described in U.S. Pat. No. 5,480,981 that retain biological activity.

[0096] This invention additionally provides for the use of CD30 antagonists in the manufacture of a medicament for the treatment of cardiovascular disease. This invention further provides for the use of polynucleotides encoding CD30 antagonists in the manufacture of a medicament for the treatment of cardiovascular disease.

[0097] E. OX40 Antagonists

[0098] Further embodiments of the present invention provide compositions and methods for the treatment of cardiovascular disease comprising one or more OX40 antagonists. A OX40 antagonist is defined herein as an entity that is capable of reducing the effective amount of endogenous OX40 ligand (OX40-L), by either partially or completely blocking the interaction of OX40-L and OX40 and thereby inhibiting OX40-mediated signaling via membrane-bound OX40, as well as partially or completely inhibiting the subsequent biological activity of activating OX40. A OX40 antagonist may bind to either OX40-L or OX40.

[0099] OX40: OX40-L biological activity, is defined herein as including, but is not limited to, binding of OX40-L to OX40 and activation of OX40; costimulatory activity on T lymphocytes; cytokine production, including IL-4; promoting the survival and proliferation of CD4+ T cells; prolongation of immune responses; enhancing effector and memory-effector T cell function by upregulating IL-2 production and increasing the life-span of effector T cells; and, enhanced tumor-specific immunity.

[0100] Such OX40 antagonists include, but are not limited to: soluble forms of OX40-L and OX40; fragments of OX40-L that bind OX40 and inhibit binding of OX40-L without activating membrane-bound OX40; fragments of OX40 that bind OX40-L and inhibit binding of OX40-L to OX40; antibodies, fusion proteins and/or peptibodies directed against OX40-L that specifically bind OX40-L and partially or completely inhibit binding of OX40-L to OX40; antibodies, fusion proteins and/or peptibodies directed against OX40 that specifically bind OX40 and inhibit binding of OX40-L without themselves activating the OX40; small molecules that bind OX40-L or OX40 and inhibit the

interaction thereof, such as OX40-L and/or OX40 peptidomimetics and/or mimotopes. As used herein, when reference is made to OX40 antagonists based on OX40-L or OX40, it is understood that the terms OX40-L and OX40 also encompass fragments, variants, muteins, derivatives and fusion proteins thereof, as described in detail below.

[0101] OX40 antagonists may comprise or be developed from OX40 polynucleotide and polypeptide sequences. The OX40 receptor, also referred to as OX40, ACT-4 and ACT35, is a protein expressed on the surface of antigen-activated mammalian CD4+ T-cells. DNA sequences encoding mouse, rat and human OX40 receptor homologs have been cloned and sequenced (see, Mallet, et al., *EMBO*, 9:1063-1068 (1990); Calderhead, et al., *J. Immunol.*, 151:5261-5271 (1993); Latza, et al., *Eur. J. Immunol.* 24:677-683 (1994); and WO 95/12673). The isolation, cloning, and characterization of human OX40 is described in U.S. Pat. Nos. 5,821,332 and 6,277,962 B1, which are incorporated herein by reference in their entirety. As mentioned above, embodiments of the present invention include anti-OX40 antibodies as OX40 antagonists. Examples of antibodies that are directed against OX40 are described in U.S. Pat. Nos. 5,821,332 and 6,277,962 B1.

[0102] OX40 proteins of the present invention include, but are not limited to, human OX40 comprising amino acids 1-277 of SEQ ID NO:28; and proteins that comprise N-terminal, C-terminal, or internal truncations of the foregoing sequences, but retain the desired biological activity. DNA sequences encoding such human OX40 polypeptides include, but are not limited to, DNA sequences comprising the nucleotide sequence of SEQ ID NO:27.

[0103] Alternative embodiments provide soluble OX40 polypeptides. Soluble OX40 polypeptides comprise all or part of the extracellular domain of a native OX40 but lack the transmembrane region that would cause retention of the polypeptide on a cell membrane. The soluble OX40 polypeptides retain the ability to bind the OX40-L. Soluble OX40 polypeptides may also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble OX40 protein is capable of being secreted. The putative signal sequence is from amino acids 1-22 or 1-24 and the extracellular domain spanning amino acids 23-212 or 24-212 or 24-212 and the transmembrane sequence spanning amino acids 213-240 of SEQ ID NO:28.

[0104] Examples of soluble OX40 polypeptides include those comprising the entire extracellular domain of a native OX40 protein or a fragment of said extracellular domain that is capable of binding OX40-L. One such soluble OX40-L polypeptides comprise amino acids z to 213 SEQ ID NO:28, wherein z is 22, 23, 24, or 25. In other words, the N-terminal amino acid of the soluble human OX40-L is selected from the amino acids in positions 22-25 of SEQ ID NO:28.

[0105] OX40 antagonists may comprise or be developed from OX40-L polynucleotide and polypeptide sequences. The isolation, cloning, preparation and characterization of human OX40-L is described in U.S. Pat. Nos. 6,156,878 and 6,242,566 B1, as well as U.S. application Ser. Nos: US 2001/0044523 A1 and US 2002/0077460 A1, which are incorporated herein by reference in their entirety. As mentioned above, embodiments of the present invention include anti-OX40-L antibodies as OX40 antagonists. Examples of

antibodies that are directed against OX40-L are described in U.S. Pat. No. 6,156,878 and 6,242,566 B1, as well as U.S. application Ser. Nos: US 2001/0044523 A1 and US 2002/0077460 A1.

[0106] OX40-L is also referred to as gp34 or ACT-4-L and is expressed on the surface of select mammalian cells, such as antigen presenting cells. Human OX40-L was initially isolated and described in Miura et al., *Mol Cell Biol* 11(3):1313-1325 (1991). U.S. Pat. No. 5,457, which is incorporated by reference in its entirety, describes the murine homologue of OX40-L.

[0107] OX40-L proteins of the present invention include, but are not limited to, human OX40-L polypeptides comprising amino acids 1-183 of SEQ ID NO:26 and polypeptides that comprise N-terminal, C-terminal, or internal truncations of the foregoing sequences, but retain the desired biological activity. DNA sequences encoding such human OX40 polypeptides include, but are not limited to, DNA sequences comprising the nucleotide sequence of SEQ ID NO:25.

[0108] Alternative embodiments provide soluble OX40-L polypeptides. Soluble OX40-L polypeptides comprise all or part of the extracellular domain of a native OX40 but lack the transmembrane region that would cause retention of the polypeptide on a cell membrane. The soluble OX40-L polypeptides retain the ability to bind the OX40. Soluble OX40-L polypeptides may also include part of the transmembrane region or part of the cytoplasmic domain or other sequences, provided that the soluble OX40-L protein is capable of being secreted.

[0109] In further embodiments, OX40 antagonists are polypeptide mimetics, such as, but not limited to peptido-mimetics, peptibodies and/or mimotopes developed from the polypeptide sequence of OX40-L (SEQ ID NO:26) and/or OX40 (SEQ ID NO:28). Polypeptide mimetics may be developed through techniques known in the art, such as combinatorial peptide libraries. Polypeptide mimetics are peptide-containing molecules which mimic elements of protein secondary structure. A OX40-L polypeptide mimetic based on the amino acid sequence of OX40-L will bind to OX40 without activating OX40 and sterically hinder binding of endogenous OX40-L. A OX40 receptor polypeptide mimetic based on the amino acid sequence of OX40 receptor will bind to OX40-L and sterically hinder binding of endogenous OX40-L to OX40. OX40-L and OX40 peptide mimetics can be used to antagonize OX40-L binding to OX40 in a subject and thereby reduce the proinflammatory effects of OX40-L. As such, OX40 antagonists in the form of polypeptide mimetics may be used to treat inflammatory and/or immunoregulatory processes associated with cardiovascular disease.

[0110] Other forms of OX40-L and OX40 that are useful in the present invention include muteins and variants (also referred to as analogs), such as naturally occurring variants, that are substantially homologous to the native OX40-L (SEQ ID NO:26) or OX40 (SEQ ID NO:28) polypeptide sequences that retain biological activity.

[0111] This invention additionally provides for the use of OX40 antagonists in the manufacture of a medicament for the treatment of cardiovascular disease. This invention further provides for the use of polynucleotides encoding OX40

antagonists in the manufacture of a medicament for the treatment of cardiovascular disease.

[0112] F. CD39

[0113] Alternative embodiments of the invention are directed to treating cardiovascular disease in a subject having cardiovascular disease comprising administering soluble CD39 polypeptides in combination with one or more IL-17 antagonists, IL-18 antagonists, 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists.

[0114] The molecular cloning and structural characterization of CD39 is presented in Maliszewski et al. (*J. Immunol.* 153:3574, 1994). A cDNA encoding the cell-surface molecule CD39 has been isolated, cloned and sequenced, as described in U.S. patent application Ser. No. 09/835,147, as well as WO 00/23459, which are incorporated by reference in their entirety. The nucleic acid sequence and predicted amino acid sequence are shown in SEQ ID NO:29 and SEQ ID NO:30, respectively.

[0115] The present invention provides methods of using soluble forms of CD39 to treat cardiovascular disease, which were constructed by removing the amino- and carboxy-terminal transmembrane domains. Soluble CD39 retains the capacity of wildtype CD39 to metabolize ATP and ADP at physiologically relevant concentrations as well as the ability to block and reverse ADP-induced platelet activation and recruitment, including platelet aggregation. The use of soluble forms of CD39 is advantageous because purification of the polypeptides from recombinant host cells is facilitated, and because soluble polypeptides are generally more suitable than membrane-bound forms for clinical administration. Because CD39 inhibits platelet activation and recruitment, and therefore platelet aggregation, the present invention provides methods and compositions for inhibiting formation of a thrombus at a site in a mammal at which platelets are inappropriately activated, methods for use in controlling platelet reactivity, thereby regulating the hemostatic and thrombotic processes, and methods of inhibiting and/or reversing platelet aggregation.

[0116] CD39 contains two putative transmembrane regions, near the amino and carboxy termini, which may serve to anchor the native protein in the cell membrane. The portion of the molecule between the transmembrane regions is external to the cell. As used herein, the term "CD39 polypeptides" includes CD39, homologs of CD39, variants, fragments, and derivatives of CD39, fusion polypeptides comprising CD39, and soluble forms of CD39 polypeptides. The CD39 gene family is reported to contain at least four human members: CD39, CD39L2, CD39L3, and CD39L4 (Chadwick and Frischauf, *Genomics* 50:357, 1998). CD39-L4 is reported to be a secreted apyrase (Mulero et al., *J. Biol. Chem.* 274(29):20064, 1999). Additional solCD39 variants have been constructed by fusing N-terminal sequences from CD39L2, CD39L3, or CD39L4 to a soluble portion of CD39, as described in detail in U.S. patent application Ser. No. 09/835,147.

[0117] CD39 is an ecto-ADPase (apyrase) located on the surface of endothelial cells. This enzyme is mainly responsible for the maintenance of blood fluidity, thus maintaining platelets in the baseline (resting) state. This is accomplished by metabolism of the major platelet agonist, adenosine diphosphate, to adenosine monophosphate, which is not an

agonist. Because ADP is the most important agonist of platelet aggregation, and is present in platelet releasate, a substance which catabolizes ADP is useful in treating or preventing disease states that involve inappropriate aggregation of platelets.

[0118] Apyrase activity resides in the extracellular domain of CD39. Thus, CD39 polypeptides include soluble forms of CD39 such as those having an amino terminus selected from the group consisting of amino acids 36-44 of SEQ ID NO:30, and a carboxy terminus selected from the group consisting of amino acids 471-478 of SEQ ID NO:30, and which exhibit CD39 biological activity. Soluble CD39 polypeptides also include those polypeptides which include part of either or both of the transmembrane regions, provided that the soluble CD39 polypeptide is capable of being secreted from a cell, and retains CD39 biological activity. Soluble CD39 polypeptides further include oligomers or fusion polypeptides comprising the extracellular portion of CD39, and fragments of any of these polypeptides that have biological activity.

[0119] The term "biological activity," with regards to CD39, includes apyrase enzymatic activity as well as the ex vivo and in vivo activities of CD39. Apyrases catalyze the hydrolysis of nucleoside tri- and/or di-phosphates, but a given apyrase may display different relative specificities for either nucleoside triphosphates or nucleoside diphosphates. Biological activity of soluble forms of CD39 may be determined, for example, in an ectonucleotidase or apyrase assay (e.g. ATPase or ADPase assays), or in an assay that measures inhibition of platelet aggregation. Exemplary assays are disclosed herein; those of skill in the art will appreciate that other, similar types of assays can be used to measure biological activity.

[0120] In further embodiments, CD39 compositions for the treatment of cardiovascular disease comprise polypeptide mimetics, such as, but not limited to peptidomimetics, peptibodies and/or mimotopes developed from the polypeptide sequence of CD39 (SEQ ID NO:30). Polypeptide mimetics may be developed through techniques known in the art, such as combinatorial peptide libraries. Polypeptide mimetics are peptide-containing molecules which mimic elements of protein secondary structure. A CD39 polypeptide mimetic based on the amino acid sequence of CD39 will catalyze the hydrolysis of nucleoside tri- and/or di-phosphates. As such, CD39 antagonists in the form of polypeptide mimetics may be used to treat cardiovascular disease.

[0121] Other forms of CD39 that are useful in the present invention include muteins and variants (also referred to as analogs), such as naturally occurring variants, that are substantially homologous to the native CD39 (SEQ ID NO:30) polypeptide sequences and as described in U.S. patent application Ser. No. 09/835,147 that retain biological activity.

[0122] This invention additionally provides for the use of CD39 in the manufacture of a medicament for the treatment of cardiovascular disease. This invention further provides for the use of polynucleotides encoding CD39 in the manufacture of a medicament for the treatment of cardiovascular disease.

[0123] CD39, in all its forms as described herein and in U.S. patent application Ser. No. 09/835,147, may be used in

combination with one or more IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist in the treatment of cardiovascular disease.

[0124] G. Further Embodiments of IL-17, IL-18, 4-1BB, CD30 and OX40 Antagonists and CD39

[0125] Other forms of the IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 that are useful in the present invention include muteins and variants (also referred to as analogs), such as naturally occurring variants, that are substantially homologous to the native sequences provided herein, as well as the sequences provided in the patents incorporated by reference.

[0126] Substantially homologous means a variant amino acid sequence that is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identical to the native amino acid sequences, as disclosed above. The percent identity of two amino acid or two nucleic acid sequences can be determined by visual inspection and mathematical calculation, or more preferably, the comparison is done by comparing sequence information using a computer program. An exemplary, preferred computer program is the Genetics Computer Group (GCG; Madison, Wis.) Wisconsin package version 10.0 program, 'GAP' (Devereux et al., 1984, *Nucl. Acids Res.* 12: 387). The preferred default parameters for the 'GAP' program includes: (1) The GCG implementation of a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted amino acid comparison matrix of Gribskov and Burgess, *Nucl. Acids Res.* 14:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Polypeptide Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; or other comparable comparison matrices; (2) a penalty of 30 for each gap and an additional penalty of 1 for each symbol in each gap for amino acid sequences, or penalty of 50 for each gap and an additional penalty of 3 for each symbol in each gap for nucleotide sequences; (3) no penalty for end gaps; and (4) no maximum penalty for long gaps. Other programs used by those skilled in the art of sequence comparison can also be used, such as, for example, the BLASTN program version 2.0.9, available for use via the National Library of Medicine website www.ncbi.nlm.nih.gov/blast2.cgi, or the UW-BLAST 2.0 algorithm. Standard default parameter settings for UW-BLAST 2.0 are described at the following Internet site: sapiens.wustl.edu/blast/blast/#Features. In addition, the BLAST algorithm uses the BLOSUM62 amino acid scoring matrix, and optional parameters that can be used are as follows: (A) inclusion of a filter to mask segments of the query sequence that have low compositional complexity (as determined by the SEG program of Wootton and Federhen (Computers and Chemistry, 1993); also see Wootton and Federhen, 1996, Analysis of compositionally biased regions in sequence databases, *Methods Enzymol.* 266: 554-71) or segments consisting of short-periodicity internal repeats (as determined by the XNU program of Claverie and States (Computers and Chemistry, 1993)), and (B) a statistical significance threshold for reporting matches against database sequences, or E-score (the expected probability of matches being found merely by chance, according to the stochastic model of Karlin and Altschul (1990); if the statistical significance ascribed to a match is greater than this E-score threshold, the match will not be reported.); preferred E-score

threshold values are 0.5, or in order of increasing preference, 0.25, 0.1, 0.05, 0.01, 0.001, 0.0001, 1e-5, 1e-10, 1e-15, 1e-20, 1e-25, 1e-30, 1e-40, 1e-50, 1e-75, or 1e-100.

[0127] Such variants include polypeptides that are substantially homologous to native IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 sequences, but which have an amino acid sequence different from that of a native IL-17 receptor because of one or more deletions, insertions or substitutions. Particular embodiments include, but are not limited to, IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptides that comprise at least one conservative amino acid substitution. Alternative embodiments comprise IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptides comprising from one to ten deletions, insertions or substitutions of amino acid residues, when compared to a native sequences. The IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 -encoding polynucleotides of the present invention include variants that differ from a native IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polynucleotide sequence because of one or more deletions, insertions or substitutions, but that encode a biologically active polypeptide. Included as variants of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptides are those variants that are naturally occurring, such as allelic forms and alternatively spliced forms, as well as variants that have been constructed by modifying the amino acid sequence of an IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptide or the nucleotide sequence of a nucleic acid encoding an IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptide.

[0128] As mentioned above, IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 variants may comprise a sequence having at least one conservatively substituted amino acid, meaning that a given amino acid residue is replaced by a residue having similar physiochemical characteristics. Alternative embodiments comprise IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 variants that comprise between 1-10, 1-20 or 1-30 conservatively substituted sequences. Generally, substitutions for one or more amino acids present in the native polypeptide should be made conservatively. Examples of conservative substitutions include substitution of amino acids outside of the active domain(s), and substitution of amino acids that do not alter the secondary and/or tertiary structure of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39. Examples of conservative substitutions include substitution of one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known. Naturally occurring variants are also encompassed by the invention. Examples of such variants are proteins that result from alternate mRNA splic-

ing events or from proteolytic cleavage of the native protein, wherein the native biological property is retained.

[0129] For example, a “conservative amino acid substitution” may involve a substitution of a native amino acid residue with a nonnative residue such that there is little or no effect on the polarity or charge of the amino acid residue at that position. Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for “alanine scanning mutagenesis” (see, for example, MacLennan et al., 1998, *Acta Physiol. Scand. Suppl.* 643:55-67; Sasaki et al., 1998, *Adv. Biophys.* 35:1-24, which discuss alanine scanning mutagenesis).

[0130] Desired amino acid substitutions (whether conservative or non-conservative) can be determined by those skilled in the art at the time such substitutions are desired. For example, amino acid substitutions can be used to identify important residues of the peptide sequence, or to increase or decrease the affinity of the peptide or vehicle-peptide molecules (see preceding formulae) described herein. Exemplary amino acid substitutions are set forth in Table 1.

TABLE 1

Amino Acid Substitutions		
Original Residues	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val, Leu, Ile	Val
Arg (R)	Lys, Gln, Asn	Lys
Asn (N)	Gln	Gln
Asp (D)	Glu	Glu
Cys (C)	Ser, Ala	Ser
Gln (Q)	Asn	Asn
Glu (E)	Asp	Asp
Gly (G)	Pro, Ala	Ala
His (H)	Asn, Gln, Lys, Arg	Arg
Ile (I)	Leu, Val, Met, Ala, Phe, Norleucine	Leu
Leu (L)	Norleucine, Ile, Val, Met, Ala, Phe	Ile
Lys (K)	Arg, 1,4 Diaminobutyric Acid, Gln, Asn	Arg
Met (M)	Leu, Phe, Ile	Leu
Phe (F)	Leu, Val, Ile, Ala, Tyr	Leu
Pro (P)	Ala	Gly
Ser (S)	Thr, Ala, Cys	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr, Phe	Tyr
Tyr (Y)	Trp, Phe, Thr, Ser	Phe
Val (V)	Ile, Met, Leu, Phe, Ala, Norleucine	Leu

[0131] In certain embodiments, conservative amino acid substitutions also encompass non-naturally occurring amino acid residues which are typically incorporated by chemical peptide synthesis rather than by synthesis in biological systems.

[0132] As noted above, naturally occurring residues may be divided into classes based on common sidechain properties that may be useful for modifications of sequence. For example, non-conservative substitutions may involve the exchange of a member of one of these classes for a member from another class. Such substituted residues may be introduced into regions of the peptide that are homologous with non-human orthologs, or into the non-homologous regions

of the molecule. In addition, one may also make modifications using P or G for the purpose of influencing chain orientation.

[0133] In making such modifications, the hydrophobic index of amino acids may be considered. Each amino acid has been assigned a hydrophobic index on the basis of their hydrophobicity and charge characteristics, these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[0134] The importance of the hydrophobic amino acid index in conferring interactive biological function on a protein is understood in the art. (Kyte, et al., *J. Mol. Biol.*, 157: 105-131 (1982)). It is known that certain amino acids may be substituted for other amino acids having a similar hydrophobic index or score and still retain a similar biological activity. In making changes based upon the hydrophobic index, the substitution of amino acids whose hydrophobic indices are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 5 are even more particularly preferred.

[0135] It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. The greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with its immunogenicity and antigenicity, i.e. with a biological property of the protein.

[0136] The following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 \pm 1); glutamate (+3.0 \pm 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 \pm 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5); tryptophan (-3.4). In making changes based upon similar hydrophilicity values, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those which are within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred. One may also identify epitopes from primary amino acid sequences on the basis of hydrophilicity. These regions are also referred to as "epitope core regions."

[0137] A skilled artisan will be able to determine suitable variants of the polypeptide as set forth in the foregoing sequences using well known techniques. For identifying suitable areas of the molecule that may be changed without destroying activity, one skilled in the art may target areas not believed to be important for activity. For example, when similar polypeptides with similar activities from the same species or from other species are known, one skilled in the art may compare the amino acid sequence of a peptide to similar peptides. With such a comparison, one can identify residues and portions of the molecules that are conserved among similar polypeptides. It will be appreciated that changes in areas of a peptide that are not conserved relative to such similar peptides would be less likely to adversely affect the biological activity and/or structure of the peptide. One skilled in the art would also know that, even in relatively conserved regions, one may substitute chemically

similar amino acids for the naturally occurring residues while retaining activity (conservative amino acid residue substitutions). Therefore, even areas that may be important for biological activity or for structure may be subject to conservative amino acid substitutions without destroying the biological activity or without adversely affecting the peptide structure.

[0138] Additionally, one skilled in the art can review structure-function studies identifying residues in similar peptides that are important for activity or structure. In view of such a comparison, one can predict the importance of amino acid residues in a peptide that correspond to amino acid residues that are important for activity or structure in similar peptides. One skilled in the art may opt for chemically similar amino acid substitutions for such predicted important amino acid residues of the peptides.

[0139] One skilled in the art can also analyze the three-dimensional structure and amino acid sequence in relation to that structure in similar polypeptides. In view of that information, one skilled in the art may predict the alignment of amino acid residues of a peptide with respect to its three dimensional structure. One skilled in the art may choose not to make radical changes to amino acid residues predicted to be on the surface of the protein, since such residues may be involved in important interactions with other molecules. Moreover, one skilled in the art may generate test variants containing a single amino acid substitution at each desired amino acid residue. The variants can then be screened using activity assays known to those skilled in the art. Such data could be used to gather information about suitable variants. For example, if one discovered that a change to a particular amino acid residue resulted in destroyed, undesirably reduced, or unsuitable activity, variants with such a change would be avoided. In other words, based on information gathered from such routine experiments, one skilled in the art can readily determine the amino acids where further substitutions should be avoided either alone or in combination with other mutations.

[0140] A number of scientific publications have been devoted to the prediction of secondary structure. See, Moult J., *Curr. Op. in Biotech.*, 7(4): 422-427 (1996); Chou et al., *Biochemistry*, 13(2): 222-245 (1974); Chou et al., *Biochemistry*, 113(2): 211-222 (1974); Chou et al., *Adv. Enzymol. Relat. Areas Mol. Biol.*, 47: 45-148 (1978); Chou et al., *Ann. Rev. Biochem.*, 47: 251-276 and Chou et al., *Biophys. J.*, 26: 367-384 (1979). Moreover, computer programs are currently available to assist with predicting secondary structure. One method of predicting secondary structure is based upon homology modeling. For example, two polypeptides or proteins which have a sequence identity of greater than 30%, or similarity greater than 40% often have similar structural topologies. The recent growth of the protein structural data base (PDB) has provided enhanced predictability of secondary structure, including the potential number of folds within a polypeptide's or protein's structure. See Holm, et al., *Nuci. Acid. Res.*, 27(1): 244-247 (1999). It has been suggested (Brenner et al., *Curr. Op. Struct. Biol.*, 7(3): 369-376 (1997)) that there are a limited number of folds in a given polypeptide or protein and that once a critical number of structures have been resolved, structural prediction will gain dramatically in accuracy.

[0141] Additional methods of predicting secondary structure include "threading" (Jones, D., *Curr. Opin. Struct. Biol.*,

7(3): 377-87 (1997); Sippl, et al., *Structure*, 4(1): 15-9 (1996)), “profile analysis” (Bowie, et al., *Science*, 253: 164-170 (1991); Gribskov, et al., *Meth. Enzym.*, 183: 146-159 (1990); Gribskov, et al., *Proc. Nat. Acad. Sci.*, 84(13): 4355-8 (1987)), and “evolutionary linkage” (See Holm, *supra*, and Brenner, *supra*).

[0142] Embodiments of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 variants include IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 variants include polypeptide sequences that are at least about 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical in amino acid sequence to the respective amino acid sequence for IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39, as described above.

[0143] Further modifications in the IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptide or IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polynucleotide sequences can be made by those skilled in the art using known techniques. Modifications of interest in the polypeptide sequences can include the alteration, substitution, replacement, insertion or deletion of a selected amino acid. For example, one or more of the cysteine residues can be deleted or replaced with another amino acid to alter the conformation of the molecule, an alteration which may involve preventing formation of incorrect intramolecular disulfide bridges upon folding or renaturation. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Pat. No. 4,518,584). As another example, N-glycosylation sites in the IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 extracellular domain can be modified to preclude glycosylation, allowing expression of a reduced carbohydrate analog in mammalian and yeast expression systems. N-glycosylation sites in eukaryotic polypeptides are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. Appropriate substitutions, additions, or deletions to the nucleotide sequence encoding these triplets will result in prevention of attachment of carbohydrate residues at the Asn side chain. Alteration of a single nucleotide, chosen so that Asn is replaced by a different amino acid, for example, is sufficient to inactivate an N-glycosylation site. Alternatively, the Ser or Thr can be replaced with another amino acid, such as Ala. Procedures for inactivating N-glycosylation sites in polypeptides are known in the art and include, for example, those described in U.S. Pat. No. 5,071,972. Additional variants within the scope of the invention include IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptides that can be modified to create derivatives thereof by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like. Covalent derivatives can be prepared by linking the chemical moieties to functional groups on amino acid side chains or at the N-terminus or C-terminus of a polypeptide. Preferably, such alteration, substitution, replacement, insertion or deletion does not diminish the biological activity of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39. One example is a variant that binds with essen-

tially the same binding affinity as does the native form. Binding affinity can be measured by conventional procedures, e.g., as described in U.S. Pat. No. 5,512,457 and as set forth herein. Furthermore, IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 molecules may be modified by the addition of one or more water-soluble polymers, such as, but not limited to, polyethylene glycol to increase bio-availability and/or pharmacokinetic half-life.

[0144] Various means for attaching chemical moieties useful for increase bio-availability and/or pharmacokinetic half-life are currently available, see, e.g., Patent Cooperation Treaty (“PCT”) International Publication No. WO 96/11953, entitled “N-Terminally Chemically Modified Protein Compositions and Methods,” herein incorporated by reference in its entirety. This PCT publication discloses, among other things, the selective attachment of water soluble polymers to the N-terminus of proteins.

[0145] A preferred polymer vehicle is polyethylene glycol (PEG). The PEG group may be of any convenient molecular weight and may be linear or branched. The average molecular weight of the PEG will preferably range from about 2 kiloDalton (“kD”) to about 100 kD, more preferably from about 5 kD to about 50 kD, most preferably from about 5 kD to about 10 kD. The PEG groups will generally be attached to the compounds of the invention via acylation or reductive alkylation through a reactive group on the PEG moiety (e.g., an aldehyde, amino, thiol, or ester group) to a reactive group on the inventive compound (e.g., an aldehyde, amino, or ester group).

[0146] A useful strategy for the PEGylation of synthetic peptides consists of combining, through forming a conjugate linkage in solution, a peptide and a PEG moiety, each bearing a special functionality that is mutually reactive toward the other. The peptides can be easily prepared with conventional solid phase synthesis. The peptides are “pre-activated” with an appropriate functional group at a specific site. The precursors are purified and fully characterized prior to reacting with the PEG moiety. Ligation of the peptide with PEG usually takes place in aqueous phase and can be easily monitored by reverse phase analytical HPLC. The PEGylated peptides can be easily purified by preparative HPLC and characterized by analytical HPLC, amino acid analysis and laser desorption mass spectrometry.

[0147] Polysaccharide polymers are another type of water-soluble polymer which may be used for protein modification. Dextrans are polysaccharide polymers comprised of individual subunits of glucose predominantly linked by α 1-6 linkages. The dextran itself is available in many molecular weight ranges, and is readily available in molecular weights from about 1 kD to about 70 kD. Dextran is a suitable water soluble polymer for use in the present invention as a vehicle by itself or in combination with another vehicle (e.g., Fc). See, for example, WO 96/11953 and WO 96/05309. The use of dextran conjugated to therapeutic or diagnostic immunoglobulins has been reported; see, for example, European Patent Publication No. 0 315 456, which is hereby incorporated by reference in its entirety. Dextran of about 1 kD to about 20 kD is preferred when dextran is used as a vehicle in accordance with the present invention.

[0148] Additional IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L

and CD39 derivatives include covalent or aggregative conjugates of the polypeptides with other polypeptides or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. Examples of fusion polypeptides are discussed below in connection with oligomers. Further, fusion polypeptides can comprise peptides added to facilitate purification and identification. Such peptides include, for example, poly-His or the antigenic identification peptides described in U.S. Pat. No. No. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988. One such peptide is the FLAG® octapeptide (SEQ ID NO:31), which is highly antigenic and provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant polypeptide. A murine hybridoma designated 4E11 produces a monoclonal antibody that binds the FLAG® peptide in the presence of certain divalent metal cations, as described in U.S. Pat. No. 5,011,912. The 4E11 hybridoma cell line has been deposited with the American Type Culture Collection under accession no. HB 9259. Monoclonal antibodies that bind the FLAG® peptide are available from Eastman Kodak Co., Scientific Imaging Systems Division, New Haven, Conn.

[0149] Additional embodiments of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 that may be used in the methods described herein include oligomers or fusion polypeptides that contain IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptide, one or more fragments of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39, or any of the derivative or variant forms of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 as disclosed herein, as well as in the U.S. patents listed above. In particular embodiments, the oligomers comprise soluble IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptides. Oligomers can be in the form of covalently linked or non-covalently-linked multimers, including dimers, trimers, or higher oligomers. In alternative embodiments, IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 oligomers comprise multiple IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptides joined via covalent or non-covalent interactions between peptide moieties fused to the polypeptides, such peptides having the property of promoting oligomerization. Leucine zippers and certain polypeptides derived from antibodies are among the peptides that can promote oligomerization of the polypeptides attached thereto, as described in more detail below.

[0150] Immunoglobulin-based Oligomers. Suitable forms of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 antagonists include chimeric proteins which include a second polypeptide that may promote the spontaneous formation by the chimeric protein of a dimer, trimer or higher order multimer that is capable of binding their respective cognates and thereby inhibiting or reducing the effects of inflammation and symptoms of cardiovascular disease. Chimeric proteins used as antagonists may be proteins that contain portions of an antibody molecule and a soluble polypeptide from IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX 40, OX40-L and/or CD39. Suitable fusion

proteins include an IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptide, e.g. the extracellular domain, or a fragment of the extracellular domain, linked to an immunoglobulin Fc region. Fragments of a Fc region may also be used, as well as Fc mureins that exhibit decreased affinity for Fc receptors. Soluble IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39, as well as fragments thereof, can be fused directly or through linker sequences to the Fc portion of an immunoglobulin.

[0151] One embodiment of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 antagonist is directed to a dimer comprising two fusion polypeptides created by fusing an IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptide to a Fc polypeptide derived from an antibody. A gene fusion encoding such a fusion polypeptide is inserted into an appropriate expression vector. IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39-Fc-fusion polypeptides are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon inter-chain disulfide bonds form between the Fc moieties to yield divalent molecules. One suitable Fc polypeptide, described in PCT application WO 93/10151, is a single chain polypeptide extending from the N-terminal hinge region to the native C-terminus of the Fc region of a human IgG1 antibody. For a bivalent form of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes can also be used to generate such fusions. For example, a polypeptide-IgM fusion would generate a decavalent form of the polypeptide of the invention.

[0152] The term "Fc polypeptide" as used herein includes native and murein forms of polypeptides made up of the Fc region of an antibody comprising any or all of the CH domains of the Fc region. Truncated forms of such polypeptides containing the hinge region that promotes dimerization are also included. Preferred Fc polypeptides comprise an Fc polypeptide derived from a human IgG1 antibody. As one alternative, an oligomer is prepared using polypeptides derived from immunoglobulins. Preparation of fusion polypeptides comprising certain heterologous polypeptides fused to various portions of antibody-derived polypeptides (including the Fc domain) are known in the art and have been described, e.g., by Ashkenazi et al. (*PNAS USA* 88:10535, 1991); Byrn et al. (*Nature* 344:677, 1990); and Hollenbaugh and Aruffo ("Construction of Immunoglobulin Fusion Polypeptides", in *Current Protocols in Immunology*, Suppl. 4, pages 10.19.1-10.19.11, 1992). Another useful Fc polypeptide is the Fc murein described in U.S. Pat. No. 5,457,035 and in Baum et al., (*EMBO J.* 13:3992-4001, 1994). The amino acid sequence of this murein is identical to that of the native Fc sequence presented in WO 93/10151, except that amino acid 19 has been changed from Leu to Ala, amino acid 20 has been changed from Leu to Glu, and amino acid 22 has been changed from Gly to Ala. The murein exhibits reduced affinity for Fc receptors. The above-described fusion polypeptides comprising Fc moieties (and oligomers formed therefrom) offer the advantage of facile purification by affinity chromatography over Polypeptide A

or Polypeptide G columns. In other embodiments, the polypeptides of the invention can be substituted for the variable portion of an antibody heavy or light chain. If fusion polypeptides are made with both heavy and light chains of an antibody, it is possible to form an oligomer with as many as four IL-17R extracellular regions.

[0153] Peptide-linker Based Oligomers. Alternatively, the oligomer is a fusion polypeptide comprising multiple IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptides, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Pat. Nos. 4,751,180 and 4,935,233. A DNA sequence encoding a desired peptide linker can be inserted between, and in the same reading frame as, the DNA sequences of the invention, using any suitable conventional technique. For example, a chemically synthesized oligonucleotide encoding the linker can be ligated between the sequences. In particular embodiments, a fusion polypeptide comprises from two to four soluble IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptides, separated by peptide linkers. Suitable peptide linkers, their combination with other polypeptides, and their use are well known by those skilled in the art.

[0154] Oligomeric forms of IL-17, IL-18, 4-1BB, CD30, OX40 and CD39 antagonists suitable for use in treating cardiovascular disease also include an IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptide, the extracellular domain of an IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptide, or an IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 antagonistic fragment of the extracellular domain associated with a zipper domain, such as zipper proteins described in U.S. Pat. No. 5,716,805, the disclosure of which is incorporated by reference herein. Other Examples of zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989), the nuclear transforming proteins, fos and jun, which preferentially form a heterodimer (O'Shea et al., *Science* 245:646, 1989; Turner and Tjian, *Science* 243:1689, 1989), and the gene product of the murine proto-oncogene, c-myc (Landschulz et al., *Science* 240:1759, 1988). The fusogenic proteins of several different viruses, including paramyxovirus, coronavirus, measles virus and many retroviruses, also possess leucine zipper domains (Buckland and Wild, *Nature* 338:547, 1989; Britton, *Nature* 353:394, 1991; Delwart and Mosialos, *AIDS Research and Human Retroviruses* 6:703, 1990). Leucine zipper domains are peptides that promote oligomerization of the polypeptides in which they are found. Leucine zippers were originally identified in several DNA-binding polypeptides and have since been found in a variety of different polypeptides. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. The zipper domain (also referred to herein as an oligomerizing, or oligomer-forming, domain) comprises a repetitive heptad repeat, often with four or five leucine residues interspersed with other amino acids. Use of leucine zippers and preparation of oligomers using leucine zippers are well known in the art.

[0155] The present invention comprises fusion polypeptides with or without spacer amino acid linking groups. For example, two soluble IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 domains can be linked with a linker sequence, such as (Gly)₄Ser(Gly)₅Ser (SEQ ID NO:32), which is described in U.S. Pat. No. 5,073,627. Other linker sequences include, for example, GlyAlaGlyGlyAlaGlySer(Gly)₅Ser (SEQ ID NO:33), (Gly₄Ser)₂ (SEQ ID NO:34), (GlyThr-Pro)₃ (SEQ ID NO:35), and (Gly₄Ser)₃Gly₄SerGly₅Ser (SEQ ID NO:36).

[0156] Nucleic acid sequences encoding soluble IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptides having altered glycosylation sites, deleted or substituted Cys residues, or modified proteolytic cleavage sites, nucleic acid sequences encoding sub-units of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptides or fusion polypeptides of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 with other peptides, allelic variants of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39, mammalian homologs of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39, and nucleic acid sequences encoding IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 polypeptides derived from alternative mRNA constructs, or those that encode peptide having substituted or additional amino acids, are examples of nucleic acid sequences according to the invention.

[0157] Due to degeneracy of the genetic code, there can be considerable variation in nucleotide sequences encoding the same amino acid sequence. Embodiments include sequences capable of hybridizing under moderately stringent conditions. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by Sambrook, Fritsch, and Maniatis (1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11; and Current Protocols in Molecular Biology, 1995, Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4), and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA. One way of achieving moderately stringent conditions involves the use of a prewashing solution containing 5×SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6×SSC, and a hybridization temperature of about 55 degrees C. (or other similar hybridization solutions, such as one containing about 50% formamide, with a hybridization temperature of about 42 degrees C.), and washing conditions of about 60 degrees C., in 0.5×SSC, 0.1% SDS. Generally, highly stringent conditions are defined as hybridization conditions as above, but with washing at approximately 68 degrees C., 0.2×SSC, 0.1% SDS. SSPE (1×SSPE is 0.15M NaCl, 10 mM NaH₂PO₄, and 1.25 mM EDTA, pH 7.4) can be substituted for SSC (1×SSC is 0.15M NaCl and 15 mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete. It should be understood that the wash temperature and wash salt concentration can be adjusted as necessary to achieve a desired

degree of stringency by applying the basic principles that govern hybridization reactions and duplex stability, as known to those skilled in the art and described further below (see, e.g., Sambrook et al., 1989). When hybridizing a nucleic acid to a target nucleic acid of unknown sequence, the hybrid length is assumed to be that of the hybridizing nucleic acid. When nucleic acids of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the nucleic acids and identifying the region or regions of optimal sequence complementarity. The hybridization temperature for hybrids anticipated to be less than 50 base pairs in length should be 5 to 10 degrees C. less than the melting temperature (T_m) of the hybrid, where T_m is determined according to the following equations. For hybrids less than 18 base pairs in length, T_m (degrees C.)=2(# of A+T bases)+4(# of #G+C bases). For hybrids above 18 base pairs in length, T_m (degrees C.)=81.5+16.6(log₁₀[Na⁺])+0.41(% G+C)−(600/N), where N is the number of bases in the hybrid, and [Na⁺] is the concentration of sodium ions in the hybridization buffer ([Na⁺] for 1×SSC=0.165M). Preferably, each such hybridizing nucleic acid has a length that is at least 15 nucleotides (or more preferably at least 18 nucleotides, or at least 20 nucleotides, or at least 25 nucleotides, or at least 30 nucleotides, or at least 40 nucleotides, or most preferably at least 50 nucleotides), or at least 25% (more preferably at least 50%, or at least 60%, or at least 70%, and most preferably at least 80%) of the length of the nucleic acid of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97.5%, or at least 99%, and most preferably at least 99.5%) with the nucleic acid of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing nucleic acids when aligned so as to maximize overlap and identity while minimizing sequence gaps as described in more detail above.

[0158] In alternative embodiments, IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polynucleotides include those that encode polypeptides that are at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99% identical in amino acid sequence to the amino acid sequence of native or at least 80% polypeptide sequences as set forth above and in the sequence listing. For polynucleotides that encode a fragment of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39, percent identity of the fragment is based on percent identity to the corresponding portion of full-length IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptide, respectively.

[0159] Mutations can be introduced into nucleic acids by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes a variant having the desired amino acid insertion, substitution, or deletion.

[0160] Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary

methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, Jan. 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and U.S. Pat. Nos. 4,518,584 and 4,737,462 disclose suitable techniques, and are incorporated by reference herein.

[0161] Polynucleotide sequences that encode IL-17 receptor polypeptides comprising various additions or substitutions of amino acid residues or sequences, or deletions of terminal or internal residues or sequences not needed for biological activity can be prepared. For example, N-glycosylation sites can be modified to preclude glycosylation while allowing expression of a homogeneous, reduced carbohydrate variant using yeast expression systems. N-glycosylation sites in eukaryotic polypeptides are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. Appropriate modifications to the nucleotide sequence encoding this triplet will result in substitutions, additions or deletions that prevent attachment of carbohydrate residues at the Asn side chain.

[0162] In another example, sequences encoding Cys residues can be altered to cause the Cys residues to be deleted or replaced with other amino acids, preventing formation of incorrect intramolecular disulfide bridges upon renaturation. Thus, Cys residues may be replaced with another amino acid or deleted without affecting polypeptide tertiary structure or disulfide bond formation.

[0163] Other approaches to mutagenesis involve modification of sequences encoding dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present. Other variants are prepared by modification of adjacent dibasic amino acid residues, to enhance expression in yeast systems in which KEX2 protease activity is present. EP 212,914 discloses the use of site-specific mutagenesis to inactivate KEX2 protease processing sites in a polypeptide. KEX2 protease processing sites are inactivated by deleting, adding or substituting residues to alter Arg-Arg, Arg-Lys, and Lys-Arg pairs to eliminate the occurrence of these adjacent basic residues. Similar modification may be made to sequences encoding sites recognized and cleaved by other proteolytic enzymes. Sub-units of a IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptide may be constructed by deleting sequences encoding terminal or internal residues or sequences not necessary for biological activity. Sequences encoding fusion polypeptides as described below may be constructed by ligating sequences encoding additional amino acid residues to the inventive sequences without affecting biological activity.

[0164] Mutations in nucleotide sequences constructed for expression of a soluble IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures such as loops or hairpins which would adversely affect translation of the receptor mRNA. Although a mutation site may be predetermined, it is not necessary that the nature of the mutation per se be predetermined. For example, in order to select for optimum

characteristics of mutants at a given site, random mutagenesis may be conducted at the target codon and the expressed mutated polypeptides screened for the desired activity.

[0165] Not all mutations in the nucleotide sequence which encodes a IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptide will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA or to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

[0166] In the genome, IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and/or CD39 polypeptides are encoded by multi-exon genes. The present invention further includes alternative mRNA constructs that can be attributed to different mRNA splicing events following transcription and which hybridize with the cDNAs disclosed herein under conditions of moderate stringency, as defined above.

[0167] H. Antibodies as IL-17, IL-18, 4-1BB, CD30 and OX40 Antagonists

[0168] IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists include antibodies that specifically bind IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40 or OX40-L. More specifically, IL-17 antagonists include antibodies directed against IL-17 that specifically bind IL-17 and partially or completely inhibit binding of IL-17 to IL-17 receptor, and antibodies directed against IL-17 receptor that specifically bind IL-17 receptor and inhibit binding of IL-17 without themselves activating the IL-17 receptor; IL-18 antagonists include antibodies directed against IL-18 that specifically bind IL-18 and partially or completely inhibit binding of IL-18 to IL-18R; antibodies directed against IL-18R that specifically bind IL-18R and inhibit receptor binding of IL-18 without themselves transducing a signal via IL-18R; 4-1BB antagonists include antibodies directed against 4-1BB that specifically bind 4-1BB and partially or completely inhibit binding of 4-1BB to 4-1BB-L; antibodies directed against 4-1BB that specifically bind 4-1BB and inhibit binding of 4-1BB-L without themselves transducing a signal via 4-1BB; CD30 antagonists include antibodies directed against CD30-L that specifically bind CD30-L and partially or completely inhibit binding of CD30-L to CD30; antibodies directed against CD30 that specifically bind CD30 and inhibit binding of CD30-L without themselves activating the CD30; and, OX40 antagonists include antibodies directed against OX40-L that specifically bind OX40-L and partially or completely inhibit binding of OX40-L to OX40; antibodies directed against OX40 that specifically bind OX40 and inhibit binding of OX40-L without themselves activating the OX40.

[0169] IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40 or OX40-L, as well as fragments, variants, muteins, derivatives and fusion proteins thereof, as set forth above, can be employed as "immuno-gens" in producing antibodies that may be used in the diagnosis and treatment of cardiovascular disease. In making IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists in the form of antibodies, when reference is made to IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30,

CD30-L, OX40 or OX40-L it is understood to also encompass fragments, variants, muteins, derivatives and fusion proteins thereof. A number of antibodies have been made to IL-17, IL-17R, IL-18, IL-18R, 4-1BB, 4-1BB-L, OX40, CD30, CD30-L and CD39, as shown in Table 3 of Example 6.

[0170] IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 contain antigenic determinants or epitopes that elicit the formation of antibodies. These antigenic determinants or epitopes can be either linear or conformational (discontinuous). Linear epitopes are composed of a single section of amino acids of the polypeptide, while conformational or discontinuous epitopes are composed of amino acids sections from different regions of the polypeptide chain that are brought into close proximity upon polypeptide folding. Epitopes can be identified by any of the methods known in the art. Additionally, epitopes from the polypeptides of the invention can be used as research reagents, in assays, and to purify specific binding antibodies from substances such as polyclonal sera or supernatants from cultured hybridomas. Such epitopes or variants thereof can be produced using techniques known in the art such as solid-phase synthesis, chemical or enzymatic cleavage of a polypeptide, or using recombinant DNA technology.

[0171] Antibodies to IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 can conveniently be generated against a recombinantly produced form of the proteins described above and provided in the respective sequence identifier numbers. IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists that are antibodies include but are not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Such antibodies can be utilized in methods of treating cardiovascular disease.

[0172] Both polyclonal and monoclonal antibodies to IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 can be prepared by conventional techniques. See, for example, *Monoclonal Antibodies, Hybridomas: A New Dimension in Biological Analyses*, Kennet et al. (eds.), Plenum Press, New York (1980); and *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988); Kohler and Milstein, (U.S. Pat. No. 4,376,110); the human B-cell hybridoma technique (Kosbor et al., *Immunology Today* 4:72, 1983; Cole et al., *Proc. Natl. Acad. Sci. USA* 80:2026, 1983); and the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies And Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96).

[0173] Methods of making humanized monoclonal antibodies are well known, and include for example those described in U.S. Pat. No. 5,585,089 (Protein Design: C L Queen et al.; "Humanized Immunoglobulins"), U.S. Pat. No. 5,565,332 ("Production of Chimeric Antibodies-A Combinatorial Approach"), U.S. Pat. No. 5,225,539 (Med Res Council: G P Winter; "Recombinant Altered Antibodies And Methods Of Making Altered Antibodies"), U.S. Pat. No. 5,693,761-762 (Protein Design: C L Queen et al.; "Polynucleotides Encoding Improved Humanized Immunoglobu-

lins", and "Humanized Immunoglobulins"), and U.S. Pat. No. 5,530,101 (Protein Design: C L Queen et al.; "Humanized Immunoglobulins"), and references cited therein.

[0174] Hybridoma cell lines that produce monoclonal antibodies specific for IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39 are contemplated herein. Such hybridomas can be produced and identified by conventional techniques. For the production of antibodies, various host animals may be immunized by injection with 1L-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD 30, CD30-L, OX40, OX40-L or CD39 polypeptide that is immunogenic. Such host animals may include, but are not limited to, horse, goat, sheep, cow, rabbits, mice, and rats, to name a few. Various adjuvants may be used to increase the immunological response. Depending on the host species, such adjuvants include, but are not limited to, Freund's (complete and incomplete), mineral 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. The monoclonal antibodies can be recovered by conventional techniques. Such monoclonal antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD, and any subclass thereof. The hybridoma producing the mAb may be cultivated in vitro or in vivo. Or, the antibody genes can be cloned and optionally otherwise altered, and expressed in another cell line approved for recombinant production of protein pharmaceuticals such as, for example, CHO cells.

[0175] Alternatively, libraries of antibody fragments can be screened and used to develop human antibodies through recombinant techniques. Such libraries are commercially available from, for example, Cambridge Antibody Technology (Melbourne, UK), and Morphosys (Munich, Del.).

[0176] In addition, techniques developed for the production of "chimeric antibodies" (Takeda et al., *Nature*, 314:452, 1985) 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 porcine mAb and a human immunoglobulin constant region. The monoclonal antibodies of the invention also include humanized versions of murine monoclonal antibodies. Such humanized antibodies can be prepared by known techniques and offer the advantage of reduced immunogenicity when the antibodies are administered to humans. For example, transgenic mice into which genetic material encoding one or more human immunoglobulin chains has been introduced may be employed. Such mice may be genetically altered in a variety of ways. The genetic manipulation may result in human immunoglobulin polypeptide chains replacing endogenous immunoglobulin chains in at least some (preferably virtually all) antibodies produced by the animal upon immunization. Procedures for the production of chimeric and further engineered monoclonal antibodies include those described in Riechmann et al. (*Nature* 332:323, 1988), Liu et al. (*PNAS* 84:3439, 1987), Larrick et al. (*Bio/Technology* 7:934, 1989), and Winter and Harris (*TIPS* 14:139, Can, 1993). Procedures to generate antibodies

transgenically can be found in GB 2,272,440, U.S. Pat. Nos. 5,569,825 and 5,545,806 and related patents claiming priority therefrom, all of which are incorporated by reference herein. For use in humans, the antibodies are typically human or humanized; techniques for creating such human antibodies are also known. Transgenic animals for making human antibodies are available from, for example, Medarex Inc. (Princeton, N.J.) Protein Design Labs, Inc. (Fremont, Calif.) and Abgenix Inc. (Fremont, Calif.).

[0177] Expression of a humanized immunoglobulin sequences in bacterial hosts may be used to select higher affinity humanized immunoglobulin sequences by mutagenizing the CDR regions and producing bacteriophage display libraries which may be screened for humanized immunoglobulin CDR variants which possess high affinity and/or high specificity binding to IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39. One potential advantage of such affinity sharpening is the generation of humanized immunoglobulin CDR variants that have improved binding affinity and/or reduced cross-reactivity with molecules other than the molecule to which they were raised. Methods for producing phage display libraries having immunoglobulin variable region sequences are provided in the art (see, e.g., Cesareni, *FEBS Lett* 307:66, 1992; Swimmer et al., *Proc. Natl. Acad. Sci. USA* 89:3756, 1992; Gram et al., *Proc. Natl. Acad. Sci. USA* 89:3576, 1992; Clackson et al., *Nature* 352:624, 1991; Scott & Smith, *Science* 249:386, 1990; Garrard et al., *Bio/Techniques* 9:1373, 1991; which are incorporated herein by reference in their entirety for all purposes. The resultant affinity sharpened CDR variant humanized immunoglobulin sequences are subsequently expressed in a suitable host.

[0178] Antibody fragments, which recognize specific epitopes, may be generated by known techniques. For example, such fragments include but are not limited to: the F(ab')₂ 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 (ab')₂ fragments. Alternatively, Fab expression libraries may be constructed (Huse et al., *Science*, 246:1275, 1989) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. Techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778; Bird, *Science* 242:423, 1988; Huston et al., *Proc. Natl. Acad. Sci. USA* 85:5879, 1988; and Ward et al., *Nature* 334:544, 1989) can also be adapted to produce single chain antibodies against polypeptides containing IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 amino acid sequences. In addition, antibodies to the IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 polypeptides can, in turn, be utilized to generate anti-idiotype antibodies using techniques known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J* 7(5):437, 1993; and Nissinoff, *J. Immunol.* 147(8):2429, 1991).

[0179] I. Nucleic Acid-Based IL-17, IL-18, 4-1BB, CD30 and OX40 Antagonists

[0180] In alternative embodiments, nucleic acid-based immuno therapy can be designed to reduce the level of endogenous IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 gene expression, e.g., using antisense or ribozyme approaches to

inhibit or prevent translation of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 mRNA transcripts; triple helix approaches to inhibit transcription of the IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 gene; or targeted homologous recombination to inactivate or “knock out” the IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 gene or its endogenous promoter.

[0181] Antisense RNA and DNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing polypeptide translation. Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are complementary to a mRNA having an IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 polynucleotide sequence. Absolute complementarity, although preferred, is not required. A sequence “complementary” to a portion of RNA, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, thereby forming a stable duplex. Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, oligonucleotides complementary to either the 5'- or 3'- non-translated, non-coding regions of the IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 gene transcript could be used in an antisense approach to inhibit translation of endogenous IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. 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, and the like. 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 Dec. 15, 1988), or hybridization-triggered cleavage agents or intercalating agents (see, e.g., Zon, 1988, Pharm. Res. 5:539-549).

[0182] The antisense molecules are delivered to cells, which express a transcript having an IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 polynucleotide sequence in vivo by, for example, injecting directly into the tissue or cell derivation site, or by use of modified antisense 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. Another approach 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 subject will result in the transcription of sufficient amounts of single

stranded RNAs that will form complementary base pairs with the endogenous IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 transcripts and thereby prevent translation of the IL-17 mRNA. For example, a vector can be introduced in vivo 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, so long as it can be transcribed to produce the desired antisense RNA. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells.

[0183] Ribozyme molecules designed to catalytically cleave mRNA transcripts having an IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 polynucleotide sequence prevent translation of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 mRNA (see, e.g., PCT International Publication WO90/11364, published Oct. 4, 1990; U.S. Pat. No. 5,824,519). Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated. There are two basic types of ribozymes namely, tetrahymena-type (Hasselhoff, *Nature*, 334:585-591, 1988) and “hammerhead”-type. Tetrahymena-type ribozymes recognize sequences, which are four bases in length, while “hammerhead”-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to tetrahymena-type ribozymes.

[0184] As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g. for improved stability, targeting, and the like). A typical method of delivery involves using a DNA construct “encoding” the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy endogenous IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 message and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

[0185] Alternatively, endogenous IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 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 IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 gene (see generally, Helene, 1991, *Anti-cancer 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).

[0186] Antisense 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 and include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides

such as, for example, solid phase phosphoramidite chemical synthesis, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, and the like). 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 supports (Sarin et al., 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:7448-7451). 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.

[0187] In alternative embodiments IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 expression may be blocked by post-translational gene silencing, such as by double-stranded RNA-induced gene silencing, also known as RNA interference (RNAi). RNA sequences of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 may be modified to provide double-stranded sequences or short hairpin RNAs for therapeutic use.

[0188] J. Screening for IL-17, IL-18, 4-1BB, CD30 and OX40 Antagonists

[0189] IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists can be evaluated using screening assays known in the art, such as high throughput test systems. The assays can be performed in a variety of formats, including protein-protein binding assays, competition binding assays, biochemical screening assays, immunoassays, cell based assays, etc. For the sake of clarity, the following examples describe exemplary assays in the context of IL-17 and IL-17R and are therefore illustrative and not limiting. The same assay formats and underlying rationale are equally applicable to IL-18:IL-18R, 4-1BB-L:4-1BB, CD30-L:CD30 and OX40-L:OX40 interactions for screening for respective antagonists.

[0190] By observing the effect that an IL-17 antagonist has on the interaction between IL-17 and IL-17 receptor in various binding assays, on IL-17/IL-17 receptor-mediated activity in functional tests, and in cell based screens, molecules that are potential therapeutics are identified because they inhibit the interaction between IL-17 and IL-17 receptor. IL-17 antagonists that partially or completely inhibit IL-17 binding to IL-17 receptor, and hence the activation of IL-17 receptor, can be useful as immunosuppressants or anti-inflammatory agents in the treatment of cardiovascular disease.

[0191] One embodiment of a screening assay that can be used to screen IL-17 antagonists for their ability to inhibit the interaction of IL-17 and IL-17 receptor comprises the steps of forming a composition comprising an IL-17 protein, an IL-17 receptor protein, and the test compound (i.e., a putative IL-17 antagonist); assaying for the level of interaction of the IL-17 protein, an IL-17 receptor protein; and comparing the level obtained in the presence of the test

compound to that obtained in the absence of the test compound, such that if the level obtained differs, a compound that affects the interaction of IL-17 and IL-17 receptor is identified. In alternative embodiments, at least one of the IL-17 or IL-17 receptor can be labeled with a detectable moiety. In alternative embodiments, one of the IL-17 or IL-17 receptor can be soluble, and the other can be bound, although alternative assay formats are possible and well known. The test compound can be added to the composition after addition of the IL-17 and IL-17 receptor, before both proteins are added, or after one protein is added and before the other is added.

[0192] In another aspect, the screening methods comprise forming a composition comprising the test compound, the IL-17 protein and cells expressing IL-17 receptor; determining the level of biological activity of IL-17 on the IL-17 receptor in the composition; and comparing the level of biological activity with that which occurs in the absence of test compound, wherein a difference in the level of biological activity indicates that the test compound affects the biological activity of the IL-17/IL-17 receptor complex. Biological activity of IL-17 on the IL-17 receptor can be assayed in any number of ways, for example but not limited to, determining the phosphorylation state of intracellular proteins (i.e., activation of the IL-17 receptor by IL-17); determining the production of proinflammatory factors, such as IL-6, IL-8, monocyte chemoattractant protein-1 and Gro α ; determining the production of hematopoietic cytokines, such as G-CSF and GM-CSF and IL-8; and determining increased expression of IL-1 β and TNF- α , as well as measuring induction of iNOS in macrophages.

[0193] A particular example of an assay for the identification of potential IL-17 antagonists is a competitive assay, which combines IL-17 and an IL-17 receptor-specific antagonist with IL-17 receptor under the appropriate conditions for a competitive assay. Either IL-17 or the IL-17 receptor-specific antagonist can be labeled so that the binding can be measured and the effectiveness of the antagonist judged. The label allows for detection by direct or indirect means. Direct means include, but are not limited to luminescence, radioactivity, optical or electron density. Indirect means include but are not limited to an enzyme or epitope tag.

[0194] Another method by which IL-17 antagonists can be identified that inhibit the interaction between IL-17 and IL-17 receptor is the solid phase method, in which IL-17 receptor is bound and placed in a medium with labeled IL-17. The amount of signal produced by the interaction between IL-17 and IL-17 receptor is measured in the presence and in the absence of a test compound. Diminished levels of signal, in comparison to a control, indicate that the test compound inhibited the interaction between IL-17 and IL-17 receptor. Increased levels of signal, in comparison to a control, indicate that the candidate molecule promotes the interaction between IL-17 and IL-17 receptor. In alternative embodiments, IL-17 could be bound and IL-17 receptor labeled. The IL-17 antagonist, IL-17 receptor and/or IL-17 proteins can be directly or indirectly labeled. For example, if the protein is recombinantly produced, one can engineer fusion proteins that can facilitate solubility, labeling, immobilization and/or detection. Fusion proteins which facilitate these processes can include, but are not limited to soluble Ig-tailed fusion proteins and His-tagged proteins. Methods

for engineering such soluble Ig-tailed fusion proteins are well known to those of skill in the art. See, for example, U.S. Pat. No. 5,116,964, and the illustrative embodiments described below. Indirect labeling involves the use of a protein, such as a labeled antibody, which specifically binds to a component of the assay.

[0195] IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists can be identified and evaluated using cells and/or cell lines derived from heart and vascular tissues. For example, cardiomyocyte cells and cell lines may be used to evaluate IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists in any of the suitable assays described herein. Biologically relevant readouts in the cardiomyocyte-based assay (or other cells) may be used to evaluate potential antagonists, such as cell survival; hypertrophic responses; and/or production of ANP and/or BNP in response to hypoxic or environmental stress.

[0196] IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists can also be identified using methods that are well suited for high-throughput screening procedures, such as scintillation proximity assays (Udenfriend et al., 1985, *Proc Natl Acad Sci USA* 82: 8672-8676), yeast two-hybrid or interaction trap assays, homogeneous time-resolved fluorescence methods (Park et al., 1999, *Anal Biochem* 269: 94-104), fluorescence resonance energy transfer (FRET) methods (Clegg R M, 1995, *Curr Opin Biotechnol* 6: 103-110), or methods that measure any changes in surface plasmon resonance when a bound polypeptide is exposed to a potential binding partner, using for example a biosensor such as that supplied by Biacore A B (Uppsala, Sweden).

[0197] Compounds that can be assayed that may also be IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists include but are not limited to small organic molecules, such as those that are commercially available—often as part of large combinatorial chemistry compound ‘libraries’—from companies such as Sigma-Aldrich (St. Louis, Mo.), Arqule (Woburn, Mass.), Enzymed (Iowa City, Iowa), Maybridge Chemical Co.(Trevillett, Cornwall, UK), MDS Panlabs (Bothell, Wash.), Pharmacopeia (Princeton, N.J.), and Trega (San Diego, Calif.). Preferred small organic molecules for screening using these assays are usually less than 10K molecular weight and can possess a number of physico-chemical and pharmacological properties which enhance cell penetration, resist degradation, and/or prolong their physiological half-lives (Gibbs, J., 1994, *Pharmaceutical Research in Molecular Oncology*, *Cell* 79(2): 193-198). Compounds including natural products, inorganic chemicals, and biologically active materials such as proteins and toxins can also be assayed using these methods for the ability to bind to serve as IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists.

[0198] Antagonizing IL-17:IL-17R, IL-18:IL-18R, 4-1BB-L:4-1BB, CD30-L:CD30 and/or OX40-L:OX40 interactions and therefore intercellular communication, cell stimulation, or immune cell activity can be manipulated by IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists to control these activities in target cells. For example, IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists or nucleic acids encoding IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists can be administered to a cell or group of cells to block IL-17:IL-17R, IL-18:IL-18R, 4-1BB-L:4-1BB, CD30-L:CD30 and/or OX40-L:OX40 binding and thereby suppress or arrest cellular communication, cell stimulation, or activity in the

target cells. In such an assay, one would determine a rate of communication or cell stimulation in the presence of the IL-17:IL-17R, IL-18:IL-18R, 4-1BB-L:4-1BB, CD30-L:CD30 and/or OX40-L:OX40 binding and then determine if such communication or cell stimulation is altered in the presence of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists. Exemplary assays for this aspect of the invention include cytokine secretion assays, T-cell co-stimulation assays, and mixed lymphocyte reactions involving antigen presenting cells and T cells. These assays are well known to those skilled in the art.

[0199] IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists may regulate cytokine, cell proliferation (either inducing or inhibiting), or cell differentiation (either inducing or inhibiting) activity, or may induce production of other cytokines in certain cell populations. Many polypeptide factors discovered to date have exhibited such activity in one or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cell stimulatory activity. The activity of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists may be evidenced by any one of a number of routine factor-dependent cell proliferation assays for cell lines including, without limitation, NF κ B, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

[0200] The activity of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists may, among other means, be measured by the following methods:

[0201] Assays for receptor-ligand activity include without limitation those described in: *Current Protocols in Immunology* Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of cellular adhesion under static conditions 7.28.1-7.28.22), Takai et al., *PNAS USA* 84:6864-6868, 1987; Bierer et al., *J. Exp. Med.* 168:1145-1156, 1988; Rosenstein et al., *J. Exp. Med.* 169:149-160 1989; Stoltenborg et al., *J. Immunol. Methods* 175:59-68, 1994; Stitt et al., *Cell* 80:661-670, 1995.

[0202] Assays for T-cell or thymocyte proliferation include without limitation those described in: *Current Protocols in Immunology*, Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (pp. 3.1-3.19: In vitro assays for mouse lymphocyte function; Chapter 7: Immunologic studies in humans); Takai et al., *J. Immunol.* 137: 3494-3500, 1986; Bertagnolli et al., *J. Immunol.* 145: 1706-1712, 1990; Bertagnolli et al., *Cellular Immunology* 133:327-341, 1991; Bertagnolli, et al., *J. Immunol.* 149:3778-3783, 1992; Bowman et al., *J. Immunol.* 152: 1756-1761, 1994.

[0203] Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Kruisbeek and Shevach, 1994, Polyclonal T cell stimulation, in *Current Protocols in Immunology*, Coligan et al. eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto; and Schreiber, 1994, Measurement of mouse and human interferon gamma in *Current Protocols in Immunology*, Coligan et al. eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto.

[0204] Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Bottomly et al., 1991, Mea-

surement of human and murine interleukin 2 and interleukin 4, in *Current Protocols in Immunology*, Coligan et al. eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto; deVries et al., *J Exp Med* 173: 1205-1211, 1991; Moreau et al., *Nature* 336:690-692, 1988; Greenberger et al., *Proc Natl Acad Sci USA* 80: 2931-2938, 1983; Nordan, 1991, Measurement of mouse and human interleukin 6, in *Current Protocols in Immunology* Coligan et al. eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto; Smith et al., *Proc Natl Acad Sci USA* 83: 1857-1861, 1986; Bennett et al., 1991, Measurement of human interleukin 11, in *Current Protocols in Immunology* Coligan et al. eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto; Ciarletta et al., 1991, Measurement of mouse and human Interleukin 9, in *Current Protocols in Immunology*; Coligan et al. eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto.

[0205] Assays for T-cell clone responses to antigens (which will identify, among others, polypeptides that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: *Current Protocols in Immunology*, Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (Chapter 3: In vitro assays for mouse lymphocyte function; Chapter 6: Cytokines and their cellular receptors; Chapter 7: Immunologic studies in humans); Weinberger et al., *PNAS USA* 77: 6091-6095, 1980; Weinberger et al., *Eur. J. Immun.* 11:405-411, 1981; Takai et al., *J. Immunol.* 137:3494-3500, 1986; Takai et al., *J. Immunol.* 140:508-512, 1988

[0206] Assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: *Current Protocols in Immunology*, Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., *PNAS USA* 78:2488-2492, 1981; Herrmann et al., *J. Immunol.* 128:1968-1974, 1982; Handa et al., *J. Immunol.* 135:1564-1572, 1985; Takai et al., *J. Immunol.* 137:3494-3500, 1986; Takai et al., *J. Immunol.* 140:508-512, 1988; Herrmann et al., *PNAS USA* 78:2488-2492, 1981; Herrmann et al., *J. Immunol.* 128:1968-1974, 1982; Handa et al., *J. Immunol.* 135:1564-1572, 1985; Takai et al., *J. Immunol.* 137:3494-3500, 1986; Bowman et al., *J. Virology* 61:1992-1998; Takai et al., *J. Immunol.* 140:508-512, 1988; Bertagnolli et al., *Cellular Immunology* 133:327-341, 1991; Brown et al., *J. Immunol.* 153:3079-3092, 1994.

[0207] Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, polypeptides that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, *J Immunol* 144: 3028-3033, 1990; and Mond and Brunswick, 1994, Assays for B cell function: in vitro antibody production, in *Current Protocols in Immunology* Coligan et al. eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto.

[0208] Mixed lymphocyte reaction (MLR) assays (which will identify, among others, polypeptides that generate predominantly Th1 and CTL responses) include, without limitation, those described in: *Current Protocols in Immunology*, Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in

Humans); Takai et al., *J. Immunol.* 137:3494-3500, 1986; Takai et al., *J. Immunol.* 140:508-512, 1988; Bertagnolli et al., *J. Immunol.* 149:3778-3783, 1992.

[0209] Dendritic cell-dependent assays (which will identify, among others, polypeptides expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., *J. Immunol.* 134:536-544, 1995; Inaba et al., *J Exp Med* 173:549-559, 1991; Macatonia et al., *J Immunol* 154:5071-5079, 1995; Porgador et al., *J Exp Med* 182:255-260, 1995; Nair et al., *J. Virology* 67:4062-4069, 1993; Huang et al., *Science* 264:961-965, 1994; Macatonia et al., *J Exp Med* 169:1255-1264, 1989; Bhardwaj et al., *J Clin Invest* 94:797-807, 1994; and Inaba et al., *J Exp Med* 172:631-640, 1990.

[0210] Assays for lymphocyte survival/apoptosis (which will identify, among others, polypeptides that prevent apoptosis after superantigen induction and polypeptides that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., *Cytometry* 13:795-808, 1992; Gorczyca et al., *Leukemia* 7:659-670, 1993; Gorczyca et al., *Cancer Research* 53:1945-1951, 1993; Itoh et al., *Cell* 66:233-243, 1991; Zacharchuk, *J Immunol* 145:4037-4045, 1990; Zamai et al., *Cytometry* 14:891-897, 1993; Gorczyca et al., *International Journal of Oncology* 1:639-648, 1992.

[0211] Assays for polypeptides that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., *Blood* 84:111-117, 1994; Fine et al., *Cell Immunol* 155:111-122, 1994; Galy et al., *Blood* 85:2770-2778, 1995; Toki et al., *PNAS USA* 88:7548-7551, 1991

[0212] Assays for embryonic stem cell differentiation (which will identify, among others, polypeptides that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al., *Cellular Biology* 15:141-151, 1995; Keller et al., *Molecular and Cellular Biology* 13:473-486, 1993; McClanahan et al., *Blood* 81:2903-2915, 1993.

[0213] Assays for cell movement and adhesion include, without limitation, those described in: *Current Protocols in Immunology* Coligan et al. eds, Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta chemokines 6.12.1-6.12.28); Taub et al. *J. Clin. Invest.* 95:1370-1376, 1995; Lind et al. *APMIS* 103:140-146, 1995; Muller et al. *Eur. J. Immunol.* 25: 1744-1748; Gruber et al. *J Immunol.* 152:5860-5867, 1994; Johnston et al. *J Immunol.* 153: 1762-1768, 1994

[0214] Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., *J. Clin. Pharmacol.* 26:131-140, 1986; Burdick et al., *Thrombosis Res.* 45:413-419, 1987; Humphrey et al., *Fibrinolysis* 5:71-79 (1991); Schaub, *Prostaglandins* 35:467-474, 1988.

II. Therapeutic Compositions and Administration thereof

[0215] This invention provides compounds, compositions, and methods for treating a subject, preferably a human patient, who is suffering from cardiovascular disease. The terms "treat", "treating", and "treatment" used herein includes curative, preventative (e.g., prophylactic) and pal-

liative or ameliorative treatment. Therapeutic compositions of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 may therefore need to be administered before, during, or after the presentation of symptoms. For therapeutic use, a soluble IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 is administered to a subject for treatment in a manner appropriate to the indication.

[0216] Embodiments of the invention include therapeutic compositions (also referred to as pharmaceutical compositions) comprising one or more soluble IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists and/or CD39. A "therapeutic composition," as used herein, comprises one or more soluble IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists and/or CD39 and a pharmaceutically acceptable diluent, preservative, solubilizer, emulsifier, adjuvant and/or carrier. As used herein, the terms "pharmaceutically" acceptable and "physiologically" acceptable are used interchangeably. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s).

[0217] Therefore, therapeutic compositions comprise all of the antagonists described in the sections above: e.g., soluble receptor molecules, ligands and/or binding proteins, such as IL-17, IL-17R, IL-18, IL-18R, IL-18 binding protein (IL-18BP), 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39, as well as biologically active fragments, muteins, variants, derivatives, fusions, etc. thereof; antibodies, fusion proteins and/or peptibodies directed against one or more of the following: IL-17, IL-17R, IL-18, IL-18R, IL-18 binding protein (IL-18BP), 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L and CD39; small molecules, such as peptidomimetics, mimotopes and the like, that antagonize the interaction between IL-17 and IL-17R, IL-18 and IL-18R, CD30-L and CD30, 4-1BB-L and 4-1BB and/or OX40-L and OX40; antisense oligonucleotides that specifically target and hybridize to the mRNA of endogenous IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 to inhibit or prevent translation of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39 mRNA transcripts; and RNA-interference molecules tailored to silence expression of IL-17, IL-17R, IL-18, IL-18R, IL-18BP, 4-1BB, 4-1BB-L, CD30, CD30-L, OX40, OX40-L or CD39.

[0218] Physiologically acceptable carriers, excipients or diluents are nontoxic to recipients at the dosages and concentrations employed. Ordinarily, preparing such compositions entails combining the IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 with buffers, antioxidants such as ascorbic acid, low molecular weight polypeptides (such as those having fewer than 10 amino acids), proteins, amino acids, carbohydrates such as glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. The IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 preferably is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in standard dosing trials, and may vary according to the chosen route of administration. In accordance with appropriate industry standards, preservatives may also be added, such as benzyl alcohol. The amount

and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the age and condition of the patient, and so forth

[0219] In one embodiment, sustained-release forms of soluble IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 described herein, are used. Sustained-release forms suitable for use in the disclosed methods include, but are not limited to, IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39, that is encapsulated in a slowly-dissolving biocompatible polymer, admixed with such a polymer, and or encased in a biocompatible semi-permeable implant. In addition, the IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 may be conjugated with polyethylene glycol (pegylated) to prolong its serum half-life or to enhance protein delivery (as described in detail above).

[0220] One type of sustained release technology that may be used in administering soluble IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 therapeutic compositions is that utilizing hydrogel materials, for example, photopolymerizable hydrogels (Sawhney et al., *Macromolecules* 26:581; 1993). Similar hydrogels have been used to prevent postsurgical adhesion formation (Hill-West et al., *Obstet. Gynecol.* 83:59, 1994) and to prevent thrombosis and vessel narrowing following vascular injury (Hill-West et al., *Proc. Natl. Acad. Sci. USA* 91:5967, 1994). Polypeptides can be incorporated into such hydrogels to provide sustained, localized release of active agents (West and Hubbel, *Reactive Polymers* 25:139, 1995; Hill-West et al., *J. Surg. Res.* 58:759; 1995). The sustained, localized release of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 when incorporated into hydrogels would be amplified by the long half life of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39.

[0221] Therapeutic compositions may be for administration for injection, or for oral, pulmonary, nasal, transdermal or other forms of administration. In general, the invention encompasses therapeutic compositions comprising effective amounts one or more IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Hyaluronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. See, e.g., *Remington's Pharmaceutical Sciences*, 18th Ed. (1990, Mack Publishing Co., Easton, Pa. 18042) pages 1435-1712 which are herein incorporated by reference in their entirety. The compositions may be prepared in liquid form, or may be in dried powder, such as lyophilized form. Implantable sustained release formulations are also contemplated, as are transdermal formulations.

[0222] Contemplated for use herein are oral solid dosage forms, which are described generally in Chapter 89 of *Remington's Pharmaceutical Sciences* (1990), 18th Ed., Mack Publishing Co. Easton Pa. 18042, which is herein incorporated by reference in its entirety. Solid dosage forms include tablets, capsules, pills, troches or lozenges, cachets or pellets. Also, liposomal or proteinoid encapsulation may be used to formulate the present compositions (as, for example, proteinoid microspheres reported in U.S. Pat. No. 4,925,673). Liposomal encapsulation may be used and the liposomes may be derivatized with various polymers (e.g., U.S. Pat. No. 5,013,556). A description of possible solid dosage forms for the therapeutic is given in Chapter 10 of Marshall, K., *Modern Pharmaceutics* (1979), edited by G. S. Bunker and C. T. Rhodes, herein incorporated by reference in its entirety. In general, the formulation will include one or more IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39, and inert ingredients which allow for protection against the stomach environment, and release of the biologically active material in the intestine.

[0223] Also specifically contemplated are oral dosage forms of the above inventive compounds. If necessary, the compounds may be chemically modified delivery is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the compound molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the compound and increase in circulation time in the body. Moieties useful as covalently attached vehicles in this invention may also be used for this purpose. Examples of such moieties include: PEG, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline. See, for example, Abuchowski and Davis, *Soluble Polymer-Enzyme Adducts, Enzymes as Drugs* (1981), Hocenberg and Roberts, eds., Wiley-Interscience, New York, N.Y., pp. 367-83; Newmark, et al. (1982), *J. Appl. Biochem.* 4:185-9. Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. Preferred for pharmaceutical usage, as indicated above, are PEG moieties. For oral delivery dosage forms, it is also possible to use a salt of a modified aliphatic amino acid, such as sodium N-(8-[2-hydroxybenzoyl]amino) caprylate (SNAC), as a carrier to enhance absorption of the therapeutic compounds of this invention. The clinical efficacy of a heparin formulation using SNAC has been demonstrated in a Phase II trial conducted by Emisphere Technologies. See U.S. Pat. No. 5,792,451, "Oral drug delivery composition and methods".

[0224] The compounds of this invention can be included in the formulation as fine multiparticulates in the form of granules or pellets of particle size about 1 mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

[0225] Colorants and flavoring agents may all be included. For example, the protein (or derivative) may be formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.

[0226] One may dilute or increase the volume of the compound of the invention with an inert material. These

diluents could include carbohydrates, especially mannitol, α -lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may also be used as fillers including calcium tripophosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

[0227] Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used as disintegrants include but are not limited to starch including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose, ultramylopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

[0228] Binders may be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

[0229] An antifrictional agent may be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to; stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

[0230] Glidants that might improve the flow properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

[0231] To aid dissolution of the compound of this invention into the aqueous environment a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents might be used and could include benzalkonium chloride or benzethonium chloride. The list of potential nonionic detergents that could be included in the formulation as surfactants are lauromacrogol 400, polyoxy 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of the protein or derivative either alone or as a mixture in different ratios.

[0232] Additives may also be included in the formulation to enhance uptake of the compound. Additives potentially having this property are for instance the fatty acids oleic acid, linoleic acid and linolenic acid.

[0233] Controlled release formulation may be desirable. The compound of this invention could be incorporated into an inert matrix which permits release by either diffusion or leaching mechanisms; e.g., gums. Slowly degenerating matrices may also be incorporated into the formulation, e.g., alginates, polysaccharides. Another form of a controlled release of the compounds of this invention is by a method based on the Oros therapeutic system (Alza Corp.), i.e., the drug is enclosed in a semipermeable membrane which allows water to enter and push drug out through a single small opening due to osmotic effects. Some enteric coatings also have a delayed release effect.

[0234] Other coatings may be used for the formulation. These include a variety of sugars which could be applied in a coating pan. The therapeutic agent could also be given in a film coated tablet and the materials used in this instance are divided into 2 groups. The first are the nonenteric materials and include methyl cellulose, ethyl cellulose, hydroxyethyl cellulose, methylhydroxy-ethyl cellulose, hydroxypropyl cellulose, hydroxypropyl-methyl cellulose, sodium carboxy-methyl cellulose, providone and the polyethylene glycols. The second group consists of the enteric materials that are commonly esters of phthalic acid.

[0235] A mix of materials might be used to provide the optimum film coating. Film coating may be carried out in a pan coater or in a fluidized bed or by compression coating.

[0236] Also contemplated herein is pulmonary delivery of the present protein (or derivatives thereof). The protein (or derivative) is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. (Other reports of this include Adjei et al., *Pharma. Res.* (1990) 7: 565-9; Adjei et al. (1990), *Internat. J. Pharmaceutics* 63: 135-44 (leuprolide acetate); Braquet et al. (1989), *J. Cardiovasc. Phannacol.* 13 (suppl.5): s.143-146 (endothelin-1); Hubbard et al. (1989), *Annals Int. Med.* 3: 206-12 ((α 1-antitrypsin); Smith et al. (1989), *J. Clin. Invest.* 84: 1145-6 ((α 1-proteinase); Oswein et al. (March 1990), "Aerosolization of Proteins", *Proc. Symp. Resp. Drug Delivery II*, Keystone, Colorado (recombinant human growth hormone); Debs et al. (1988), *J. Immunol.* 140: 3482-8 (interferon- γ and tumor necrosis factor α) and Platz et al., U.S. Pat. No. 5,284,656 (granulocyte colony stimulating factor).

[0237] Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art. Some specific examples of commercially available devices suitable for the practice of this invention are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Mo.; the Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colo.; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, N.C.; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Mass.

[0238] All such devices require the use of formulations suitable for the dispensing of the inventive compound. Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to diluents, adjuvants and/or carriers useful in therapy.

[0239] The inventive compound should most advantageously be prepared in particulate form with an average particle size of less than 10 μm (or microns), most preferably 0.5 to 5 μm , for most effective delivery to the distal lung.

[0240] Pharmaceutically acceptable carriers include carbohydrates such as trehalose, mannitol, xylitol, sucrose, lactose, and sorbitol. Other ingredients for use in formulations may include DPPC, DOPE, DSPC and DOPC. Natural or synthetic surfactants may be used. PEG may be used (even apart from its use in derivatizing the protein or analog). Dextrans, such as cyclodextran, may be used. Bile salts and other related enhancers may be used. Cellulose and cellulose derivatives may be used. Amino acids may be used, such as use in a buffer formulation.

[0241] Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated.

[0242] Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise the inventive compound dissolved in water at a concentration of about 0.1 to 25 mg of biologically active protein per mL of solution. The formulation may also include a buffer and a simple sugar (e.g., for protein stabilization and regulation of osmotic pressure). The nebulizer formulation may also contain a surfactant, to reduce or prevent surface induced aggregation of the protein caused by atomization of the solution in forming the aerosol.

[0243] Formulations for use with a metered-dose inhaler device will generally comprise a finely divided powder containing the inventive compound suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant.

[0244] Formulations for dispensing from a powder inhaler device will comprise a finely divided dry powder containing the inventive compound and may also include a bulking agent, such as lactose, sorbitol, sucrose, mannitol, trehalose, or xylitol in amounts which facilitate dispersal of the powder from the device, e.g., 50 to 90% by weight of the formulation.

[0245] Nasal delivery of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 is also contemplated. Nasal delivery allows the passage of the protein to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran. Delivery via transport across other mucous membranes is also contemplated.

[0246] In practicing the method of treatment or use of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39, a therapeutically effective amount is administered to a subject. As used herein, the term "therapeutically effective amount" means the total amount of each therapeutic composition that is sufficient to show a meaningful patient benefit, i.e., treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of

treatment, healing, prevention or amelioration of such conditions. When applied to an individual therapeutic composition, administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously. As used herein, the phrase "administering a therapeutically effective amount" of a therapeutic agent means that the patient is treated with said therapeutic composition in an amount and for a time sufficient to induce an improvement, and preferably a sustained improvement, in at least one indicator that reflects the severity of the disorder. An improvement is considered "sustained" if the patient exhibits the improvement on at least two occasions separated by one or more days, or more preferably, by one or more weeks. The degree of improvement is determined based on signs or symptoms, and determinations can also employ questionnaires that are administered to the patient, such as quality-of-life questionnaires. Various indicators that reflect the extent of the patient's illness can be assessed for determining whether the amount and time of the treatment is sufficient. The baseline value for the chosen indicator or indicators is established by examination of the patient prior to administration of the first dose of the therapeutic agent. Preferably, the baseline examination is done within about 60 days of administering the first dose. If the therapeutic agent is being administered to treat acute symptoms, the first dose is administered as soon as practically possible after the injury has occurred. Improvement is induced by administering therapeutic compositions such as IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 until the patient manifests an improvement over baseline for the chosen indicator or indicators. In treating chronic conditions, this degree of improvement is obtained by repeatedly administering this medicament over a period of at least a month or more, e.g., for one, two, or three months or longer, or indefinitely. A period of one to six weeks, or even a single dose, often is sufficient for treating injuries or other acute conditions. Although the extent of the patient's illness after treatment may appear improved according to one or more indicators, treatment may be continued indefinitely at the same level or at a reduced dose or frequency. Once treatment has been reduced or discontinued, it later may be resumed at the original level if symptoms should reappear.

[0247] One skilled in the pertinent art will recognize that suitable dosages will vary, depending upon such factors as the nature and severity of the disorder to be treated, the patient's body weight, age, general condition, and prior illnesses and/or treatments, and the route of administration. Preliminary doses can be determined according to animal tests, and the scaling of dosages for human administration is performed according to art-accepted practices such as standard dosing trials. For example, the therapeutically effective dose can be estimated initially from cell culture assays. The dosage will depend on the specific activity of the compound and can be readily determined by routine experimentation. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture, while minimizing toxicities. Such information can be used to more accurately determine useful doses in humans. Ultimately, the attending physician will

decide the amount of polypeptide of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of polypeptide of the present invention and observe the patient's response. Larger doses of polypeptide of the present invention can be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various therapeutic compositions used to practice the method of the present invention should contain about 0.01 ng to about 100 mg (preferably about 0.1 ng to about 10 mg, more preferably about 0.1 microgram to about 1 mg) of polypeptide of the present invention per kg body weight. In one embodiment of the invention, IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 are administered one time per week to treat the various medical disorders disclosed herein, in another embodiment is administered at least two times per week, and in another embodiment is administered at least three times per week. If injected, the effective amount of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 per adult dose ranges from 1-20 mg/m², and preferably is about 5-12 mg/m². Alternatively, a flat dose can be administered, whose amount may range from 5-100 mg/dose. Exemplary dose ranges for a flat dose to be administered by subcutaneous injection are 5-25 mg/dose, 25-50 mg/dose and 50-100 mg/dose. In one embodiment of the invention, the various indications described below are treated by administering a preparation acceptable for injection containing IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 at 25 mg/dose, or alternatively, containing 50 mg per dose. The 25 mg or 50 mg dose can be administered repeatedly, particularly for chronic conditions. If a route of administration other than injection is used, the dose is appropriately adjusted in accord with standard medical practices. In many instances, an improvement in a patient's condition will be obtained by injecting a dose of about 25 mg of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 one to three times per week over a period of at least three weeks, or a dose of 50 mg of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39 one or two times per week for at least three weeks, though treatment for longer periods may be necessary to induce the desired degree of improvement. For incurable chronic conditions, the regimen can be continued indefinitely, with adjustments being made to dose and frequency if such are deemed necessary by the patient's physician. The foregoing doses are examples for an adult patient who is a person who is 18 years of age or older. For pediatric patients (age 4-17), a suitable regimen involves the subcutaneous injection of 0.4 mg/kg, up to a maximum dose of 25 mg of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist and/or CD39, administered by subcutaneous injection one or more times per week. If an IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonist is in the form of an antibody, a preferred dose range is 0.1 to 20 mg/kg, and more preferably is 1-10 mg/kg. Another embodiment of a dose range is 0.75 to 7.5 mg/kg of body weight. Humanized antibodies are preferred, that is, antibodies in which only the antigen-binding portion of the antibody molecule is derived from a non-human source. Such antibodies can be injected or administered intravenously.

III. Therapeutic Applications

[0248] IL-17, IL-18, 4-1BB, CD30 and OX40 antagonists may be used to treat cardiovascular disease. Embodiments of the present invention include methods of treating cardiovascular disease in a subject having having cardiovascular disease comprising administering an effective amount of one or more IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists and/or CD39, alone or in any combination.

[0249] Cardiovascular disease includes disease states having pathophysiology of the heart and vasculature systems, as well as organs and systems compromised by disease states of the heart and vasculature systems. Examples include, but are not limited to: inflammation of the heart and/or vasculature such as myocarditis, chronic autoimmune myocarditis, bacterial and viral myocarditis, as well as infective endocarditis; heart failure; congestive heart failure; chronic heart failure; cachexia of heart failure; cardiomyopathy, including non-ischemic (dilated cardiomyopathy; idiopathic dilated cardiomyopathy; cardiogenic shock, heart failure secondary to extracorporeal circulatory support ("post-pump syndrome"), heart failure following ischemia/reperfusion injury, brain death associated heart failure (as described in Owen et al., 1999 (Circulation. 1999 May 18;99(19):2565-70)); hypertrophic cardiomyopathy; restrictive cardiomyopathy; non-ischemic systemic hypertension; valvular disease; arrhythmogenic right ventricular cardiomyopathy) and ischemic (atherogenesis; atherosclerosis; arteriosclerosis; peripheral vascular disease; coronary artery disease; infarctions, including stroke, transient ischemic attacks and myocardial infarctions). Additional disease states encompassed by the definition of cardiovascular disease include: aneurysms; arteritis; angina; embolism; platelet-associated ischemic disorders; ischemia/reperfusion injury; restenosis; mitral and/or tricuspid regurgitation; mitral stenosis; silent myocardial ischemia; Raynaud's phenomena; thrombosis; deep venous thrombosis; pulmonary embolism; thrombotic microangiopathies including thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS), essential thrombocythemia, disseminated intravascular coagulation (DIC), and thrombosis and coagulopathies associated with exposure to a foreign or injured tissue surface; thrombophlebitis; vasculitis, including Kawasaki's vasculitis; Takayasu's arteritis; veno-occlusive disease, giant cell arteritis, Wegener's granulomatosis; Schoenlein-Henoch purpura, as well as cardiovascular disease arising from periodontal infections by one or more oral pathogens, such as bacteria.

[0250] Additional examples of the therapeutic uses of one or more IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists alone or in combination with CD39 include the treatment of individuals who suffer from coronary artery disease or injury following platelet-associated ischemic disorders including lung ischemia, coronary ischemia, and cerebral ischemia, and for the prevention of reocclusion following thrombosis, thrombotic disorders including coronary artery thrombosis, cerebral artery thrombosis, intracardiac thrombosis, peripheral artery thrombosis, venous thrombosis, thrombotic microangiopathies including thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS), essential thrombocythemia, disseminated intravascular coagulation (DIC), and thrombosis and coagulopathies associated with exposure to a foreign or injured tissue surface, in combination with angioplasty, carotid endarter-

ectomy, anastomosis of vascular grafts, and chronic cardiovascular devices such as in-dwelling catheters or shunts.

[0251] Further indications include subjects that are or will be undergoing angioplasty procedures (i.e., balloon angioplasty, laser angioplasty, coronary atherectomy and similar techniques), placement of endovascular prosthetic devices such as carotid, coronary, peripheral arterial or other endovascular stents, dialysis access devices, or procedures to treat peripheral vascular disease; individuals undergoing surgery that has a high risk of thrombus formation (i.e., coronary bypass surgery, insertion of a prosthetic valve or vessel and the like).

[0252] In addition, IL-17 and/or IL-18 are prognostic indicators of cardiovascular disease and disease severity. IL-17 and/or IL-18 are also prognostic indicators of donor adequacy and post-transplant outcome. Therefore, further embodiments of the invention include assays for measuring IL-17 and/or IL-18 levels in subjects being screened for cardiovascular disease, cardiovascular disease severity, donor adequacy and post-transplant outcome.

[0253] Various indicators that reflect the extent of the patient's illness may be assessed for determining whether the amount and time of the treatment is sufficient. The baseline value for the chosen indicator or indicators is established by examination of the patient prior to administration of the first dose of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists alone or in combination with soluble CD39. Preferably, the baseline examination is done within about 60 days of administering the first dose.

[0254] Improvement is induced by repeatedly administering a dose of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists alone or in combination with soluble CD39 until the patient manifests an improvement over baseline for the chosen indicator or indicators. In treating chronic conditions, this degree of improvement is obtained by repeatedly administering this medicament over a period of at least a month or more, e.g., for one, two, or three months or longer, or indefinitely. A period of one to six weeks, or even a single dose, often is sufficient for treating acute conditions.

[0255] Although the extent of the patient's illness after treatment may appear improved according to one or more indicators, treatment may be continued indefinitely at the same level or at a reduced dose or frequency. Once treatment has been reduced or discontinued, it later may be resumed at the original level if symptoms should reappear.

[0256] Therapeutic compositions of the invention may be administered alone or in combination with a therapeutically effective amount of other drugs. The invention includes the administration of IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists alone or in combination with soluble CD39 concurrently with one or more other drugs that are administered to the same patient in combination with the IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists and/or CD39, each drug being administered according to a regimen suitable for that medicament. "Concurrent administration" encompasses simultaneous or sequential treatment with the components of the combination, as well as regimens in which the drugs are alternated, or wherein one component is administered long-term and the other(s) are administered intermittently. Components can be administered in the same or in separate compositions, and by the same or different routes of administration.

[0257] Examples of other drugs or therapeutic compositions that may be used in combination with IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists alone or in combination with soluble CD39 include: analgesic agents, disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory drugs (NSAIDs), and any immune and/or inflammatory modulators. Non-steroidal anti-inflammatories may include, but are not limited to: salicylic acid (aspirin); ibuprofen; indomethacin; celecoxib; rofecoxib; ketorolac; nambumetone; piroxicam; naproxen; oxaprozin; sulindac; ketoprofen; diclofenac; other COX-1 and/or COX-2 inhibitors, salicylic acid derivatives, propionic acid derivatives, acetic acid derivatives, fumaric acid derivatives, carboxylic acid derivatives, butyric acid derivatives, oxycams, pyrazoles and pyrazolones, including newly developed anti-inflammatories.

[0258] Therapeutic compositions of this invention may be administered with one or more of the following: modulators of other members of the TNF/TNF receptor family, including TNF antagonists, such as etanercept (Enbrel™), sTNF-RI, onercept, D2E7, and Remicade™; IL-1 inhibitors, including IL-1ra molecules such as anakinra and more recently discovered IL-1ra-like molecules such as IL-1Hy1 and IL-1Hy2; IL-1 “trap” molecules as described in U.S. Pat. No. 5,844,099; IL-1 antibodies; solubilized IL-1 receptor, and the like; IL-6 inhibitors (e.g., antibodies to IL-6); IL-8 inhibitors (e.g., antibodies to IL-8); Interleukin-1 converting enzyme (ICE) modulators; insulin-like growth factors (IGF-1, IGF-2) and modulators thereof; transforming growth factor- β (TGF- β), TGF- β family members, and TGF- β modulators; fibroblast growth factors FGF-1 to FGF-10, and FGF modulators; COX-2 inhibitors, such as Celebrex™ and Vioxx™; prostaglandin analogs (e.g., E series prostaglandins); matrix metalloproteinase (MMP) modulators; nitric oxide synthase (NOS) modulators, including modulators of inducible NOS; modulators of glucocorticoid receptor; modulators of glutamate receptor; modulators of lipopolysaccharide (LPS) levels; anti-cancer agents, including inhibitors of oncogenes (e.g., fos, jun) and interferons; noradrenaline and modulators and mimetics thereof.

[0259] Additional embodiments of compositions that can be administered concurrently with the pharmaceutical compositions of the invention are: cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, Flt3-Ligand, TNF, IL-1, IL-2, IL-3, IL4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin, or inhibitors or antagonists of any of these factors. The pharmaceutical composition can further contain other agents which either enhance the activity of the polypeptide or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with polypeptide of the invention, or to minimize side effects. Conversely, IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists and/or soluble CD39 may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent.

[0260] Further embodiments of drugs to be administered concurrently include but are not limited to antivirals, antibiotics, analgesics, corticosteroids, antagonists of inflammatory cytokines, non-steroidal anti-inflammatories, pentoxifylline, thalidomide, and disease-modifying antirheumatic drugs (DMARDs) such as azathioprine, cyclophosphamide, cyclosporine, hydroxychloroquine sulfate, methotrexate, leflunomide, minocycline, penicillamine, sulfasalazine and gold compounds such as oral gold, gold sodium thiomalate, and aurothioglucose.

[0261] Of course, IL-17, IL-18, 4-1BB, CD30 and/or OX40 antagonists and/or soluble CD39, as well as other therapeutic compositions described above, may be administered in conjunction with other recognized therapies or treatments, such as any surgical procedures involving the heart and vasculature (coronary bypass, heart transplant, valve replacement, angioplasty, stenting, atherectomy, aortic aneurysm repair, valve plication, ventricular assist device insertion, ventricular volume reduction surgery, any form of peripheral arterial surgery including bypass, vessel recanalisation or reconstruction, pediatric cardiovascular surgery including repair and correction of complex congenital lesions); Lipid-lowering drugs (such as, but not limited to Lipitor, simvastatin, pravastatin, atorvastatin, non-HMG CoA reductase inhibitors); blood pressure-regulating drugs (including but not limited to calcium channel antagonists, ACE-inhibitors, beta-blockers, orally and systemically available nitric oxide donors such as GTN); angiotensin-converting enzyme inhibitors, and peroxisome proliferator-activated receptor ligands.

[0262] In other embodiments of the invention, 4-1BB, CD30 and/or OX40 antagonists may be used to prevent, reduce and/or ameliorate the cardiotoxicity of chemotherapeutics. Drug toxicity remains a significant barrier to the delivery of curative doses of cancer chemotherapy. Many chemotherapeutic drugs cause direct injury to the heart, either acutely in the form of myocardial tissue injury or dysrhythmias, or in a chronic fashion associated with congestive heart failure. Examples of acute cardiotoxicity include supraventricular tachyarrhythmias, which may be associated ECG changes, such as ST-T segment changes, decreased voltage, T-wave flattening, as well as atrial and ventricular ectopy. Acute effects occur in up to 40% of patients receiving bolus doxorubicin and are usually transient. Chronic anthracycline cardiotoxicity may be manifested as arrhythmias, myocarditis, pericarditis, myocardial infarction and cardiomyopathy that is dose- and schedule-dependent. Above cumulative bolus doses of 550 mg/m² the risk of congestive heart failure increases rapidly. Doses of less than 450 mg/m² pose a risk of less than 10%. Patients receiving anthracyclines also demonstrate late-appearing cardiac toxicity occurring greater than 5 years after exposure to doxorubicin. Cardiac dysfunction is manifested as congestive heart failure or dysrhythmias and can occur in patients that were previously asymptomatic. It is estimated that approximately 5% of patients surviving ten years after exposure to doxorubicin will experience this toxicity (see, Page, R., *Cancer Management: A Multidisciplinary Approach*, PRR Inc., Fifth Edition (2001)).

[0263] Principal among the cardiotoxic agents are cytostatic antibiotics of the anthracycline class. The class of anthracyclines includes, but is not limited to, Adriamycin (Doxorubicin), Daunorubicin, Ellence (Epirubicin), Idar-

bicin, Mitroxantrone, and the like. Therefore, embodiments of the invention provide methods of preventing, reducing and/or ameliorating the cardiotoxic effects of anthracyclines comprising administrating an effective amount of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists. Embodiments of the invention also provide compositions for preventing, reducing and/or ameliorating the cardiotoxic effects of anthracyclines comprising an antagonist selected from the group consisting of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists.

[0264] Embodiments of the invention provide methods of preventing, reducing and/or ameliorating the cardiotoxic effects of anthracyclines selected from the group consisting of Adriamycin (Doxorubicin), Daunorubicin, Ellence (Epirubicin), Idarubicin and Mitroxantrone, comprising administering an effective amount of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists. Embodiments of the invention also provide compositions for preventing, reducing and/or ameliorating the cardiotoxic effects of anthracyclines selected from the group consisting of Adriamycin (Doxorubicin), Daunorubicin, Ellence (Epirubicin), Idarubicin and Mitroxantrone, comprising an antagonist selected from the group consisting of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists.

[0265] 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists may be used to prevent, reduce and/or ameliorate the cardiotoxic effects of other chemotherapeutics having cardiotoxicity, such as, but not limited to: Amsacrine, Busulfan, Cisplatin, Cyclophosphamide, Fluorouracil, Herceptin (and other Her2/neu-targeted modalities), Ifosfamide, Interferons, Interleukin-2, Mitomycin, Paclitaxel, Vinblastine, Vincristine and Xeloda (capecitabine).

[0266] Therefore, embodiments of the invention provide methods of preventing, reducing and/or ameliorating the cardiotoxic effects of chemotherapeutics having cardiotoxic side effects, comprising administrating an effective amount of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists. Embodiments of the invention also provide compositions for preventing, reducing and/or ameliorating the cardiotoxic effects of chemotherapeutics having cardiotoxic side effects comprising an antagonist selected from the group consisting of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists.

[0267] Embodiments of the invention provide methods of preventing, reducing and/or ameliorating the cardiotoxic effects of chemotherapeutics selected from the group consisting of Amsacrine, Busulfan, Cisplatin, Cyclophosphamide, Fluorouracil, Herceptin (and other Her2/neu-targeted modalities), Ifosfamide, Interferons, Interleukin-2, Mitomycin, Paclitaxel, Vinblastine, Vincristine and Xeloda (capecitabine), comprising administrating an effective amount of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists. Embodiments of the invention also provide compositions for preventing, reducing and/or ameliorating the cardiotoxic effects of chemotherapeutics selected from the group consisting of Amsacrine, Busulfan, Cisplatin, Cyclophosphamide, Fluorouracil, Herceptin (and other Her2/neu-targeted modalities), Ifosfamide, Interferons, Interleukin-2, Mitomycin, Paclitaxel, Vinblastine, Vincristine and Xeloda (capecitabine), comprising an antagonist selected from the group consisting of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists.

[0268] Embodiments of the invention provide methods of treating cancer in a subject in need thereof, wherein the dosage of a chemotherapeutic having cardiotoxicity is increased to more effectively treat the cancer but the cardiotoxic effects of the chemotherapeutic is prevented, reduced and/or ameliorated by administering an antagonist selected from the group consisting of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists. Embodiments of the invention provide methods of treating cancer in a subject in need thereof, wherein the dosage of an anthracycline is increased to more effectively treat the cancer but the cardiotoxic effects of the anthracycline is prevented, reduced and/or ameliorated by administering an antagonist selected from the group consisting of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists. Embodiments of the invention provide methods of treating cancer in a subject in need thereof, wherein the dosage of an anthracycline selected from the group consisting of Adriamycin (Doxorubicin), Daunorubicin, Ellence (Epirubicin), Idarubicin and Mitroxantrone is increased to more effectively treat the cancer but the cardiotoxic effects of the anthracycline is prevented, reduced and/or ameliorated by administering an antagonist selected from the group consisting of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists. Embodiments of the invention provide methods of treating cancer in a subject in need thereof, wherein the dosage of a chemotherapeutic selected from the group consisting of Amascrine, Busulfan, Cisplatin, Cyclophosphamide, Fluorouracil, Herceptin (and other Her2/neu-targeted modalities), Ifosfamide, Interferons, Interleukin-2, Mitomycin, Paclitaxel, Vinblastine, Vincristine and Xeloda (capecitabine) is increased to more effectively treat the cancer but the cardiotoxic effects of the chemotherapeutic is prevented, reduced and/or ameliorated by administering an antagonist selected from the group consisting of a 4-1BB antagonists, CD30 antagonists and/or OX40 antagonists.

[0269] The present invention is not to be limited in scope by the specific embodiments described herein, 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.

[0270] The invention having been described, the following examples are offered by way of illustration, and not limitation.

Sequence Identity Numbers and Associated Molecules

SEQ ID NO.	Molecule
1	IL-17 polynucleotide sequence
2	IL-17 amino acid sequence
3	IL-17 Receptor polynucleotide sequence
4	IL-17 Receptor amino acid sequence
5	IL-18 Receptor: IL-1Rrp1 polynucleotide sequence
6	IL-18 Receptor: IL-1Rrp1 amino acid sequence
7	IL-18 Receptor: AcPL polynucleotide sequence
8	IL-18 Receptor: AcPL amino acid sequence
9	IL-18 Binding Protein a polynucleotide sequence

-continued

<u>Sequence Identity Numbers and Associated Molecules</u>	
SEQ ID NO.	Molecule
10	IL-18 Binding Protein a amino acid sequence
11	IL-18 Binding Protein-Fc fusion amino acid sequence
12	IL-18 polynucleotide sequence (unprocessed)
13	IL-18 amino acid sequence (unprocessed)
14	IL-18 amino acid sequence (ICE-processed)
15	4-1BB-L polynucleotide sequence
16	4-1BB-L amino acid sequence
17	4-1BB polynucleotide sequence
18	4-1BB amino acid sequence
19	CD30-L polynucleotide sequence (nt 1-648)
20	CD30-L polypeptide sequence (aa 1-215)
21	CD30-L polynucleotide sequence (nt 1-705)
22	CD30-L polypeptide sequence (aa 1-234)
23	CD30 polynucleotide sequence
24	CD30 polypeptide sequence
25	OX40-L polynucleotide sequence
26	OX40-L polypeptide sequence
27	OX40 polynucleotide sequence
28	OX40 polypeptide sequence
29	CD39 polynucleotide sequence
30	CD39 polypeptide sequence
31	Flag ® octapeptide
32	Linker - (Gly) ₄ Ser(Gly) ₅ Ser
33	Linker - GlyAlaGlyGlyAlaGlySer(Gly) ₅ Ser
34	Linker - (Gly ₂ Ser) ₂
35	Linker - (GlyThrPro) ₃
36	Linker - (Gly ₄ Ser) ₃ Gly ₄ SerGly ₅ Ser

EXAMPLES

Example 1

IL-17 and IL-18 Plasma Levels are Elevated in Cardiomyopathy Patients

[0271] These studies demonstrate IL-17 and IL-18 are elevated in human patients having various forms and severity of cardiovascular disease.

[0272] In a series of studies, plasma levels of IL-17 and IL-18 were found to be elevated in patients having acute and chronic heart failure. Plasma from brain-dead organ donors was obtained at the time of heart removal and stored. The clinical outcome of the recipient that received the various organs was noted. A group of recipients that survived and did well was collected along with a group that died within 72 hours of transplantation due to cardiac failure refractory to maximal medical support. The plasma from the original donors, recipients and unused donors having an ejection fraction (EF) of less than 30% was assayed for IL-17 and IL-18. Cytokine levels were measured in plasma samples essentially as described in the protocols provided in commercially available ELISA kits (see, for example, QUANT-KINE® R&D Systems, Minneapolis, Minn., which provides assays for the quantitative determination of human IL-17 and human IL-18).

[0273] As shown in **FIGS. 1A and 1B**, IL-17 and IL-18 were elevated in patients that died shortly after transplantation in contrast to patients that survived. IL-17 and IL-18 were also elevated in unused donors having an ejection fraction (EF) of less than 30%, suggesting a correlation between circulating IL-17 and IL-18 levels and disease severity. This study further shows the diagnostic and prog-

nostic value of assaying for IL-17 and IL-18 cytokine levels in heart patients, such as for the assessment of post-transplant survival.

[0274] Using the samples described above, IL-18 receptor expression was assessed. Heart samples were homogenized in ice-cold lysis buffer (New England Biolabs, Beverly, Mass.). The homogenate was centrifuged at 4° C. (12000×5 mins) and the supernatant assayed for protein content (Pierce BCA kit). The same total amount of protein (20 mg/lane) was subjected to SDS-PAGE using 5% gels. Proteins were then transferred to nitrocellulose membrane and IL-18 receptor visualized using a polyclonal antibody raised against IL-18R alpha (AF840-R&D Systems, Minneapolis, Minn.), and the ECL kit.

[0275] As shown in **FIG. 2** the relative abundance of IL-18 receptor is higher in patients having an ejection fraction (EF) of less than 30% as compared to patients in end stage heart failure-ESF (i.e., NYHA stage 4 cardiomyopathy) and patients having an ejection fraction of greater than 60%. These data demonstrate that elevated IL-18 receptor expression is associated with impaired myocardial function. Given that IL-18 signals through the Toll-IL-1 receptor pathway (TIR) and that both lipopolysaccharide and IL-1 are negatively inotropic, elevated expression of the IL-18 receptor could well account for part of the myocardial dysfunction seen in these patients.

[0276] Samples from cardiac patients participating in the multi-center Renaissance Trial were evaluated. The patients exhibited a continuum of functional capacity and objective evidence of cardiovascular disease, as classified by the Criteria Committee of the New York Heart Association (*Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels*. 9th ed. Boston Mass: Little, Brown & co; 1994:253-256). Samples were evaluated for cytokine levels using the assays described above.

[0277] FIGS. 3A-3D depict the plasma concentration of IL-17 and IL-18 (pg/ml) in cardiomyopathy patients diagnosed in NYHA classes 1, 2, 3a, 3b and 4. This data shows a dramatic and unexpected increase in the amount of circulating IL-17 in NYHA classes 2, 3a and 3b (**FIG. 1A**). Elevated IL-17 levels were found in both non-ischemic and ischemic cardiomyopathy for the same classes (**FIG. 1B**). IL-17 levels were significantly higher in NYHA class 3a for ischemic cardiomyopathy. Significantly, this data shows a direct correlation between IL-17 levels and progression of disease up to NYHA class 3b and a decrease in IL-17 in NYHA class 4.

[0278] Plasma levels of IL-18 were also elevated in NYHA classes 2, 3a, 3b and 4 (**FIG. 3C**). Similar to IL-17, this data shows a direct correlation between IL-18 levels and progression of disease up to NYHA class 3b with a decrease in IL-18 in NYHA class 4 (**FIG. 3C**). When broken down into non-ischemic and ischemic cardiomyopathy, IL-18 levels were elevated in both non-ischemic and ischemic cardiomyopathy for the same NYHA classes (**FIG. 3D**). Non-ischemic cardiomyopathy patients had comparatively higher levels of IL-18 for NYHA classes 2, 3a and 3b.

[0279] This data demonstrates that IL-17 plasma levels can be used as a prognostic indicator of cardiovascular disease and disease severity. Without being bound by theory, the relative expression of IL-17 and IL-18 may be diagnostic

of non-ischemic versus ischemic cardiomyopathy (compare **FIGS. 3B and 3D**). Therefore, assays to detect circulating levels of IL-17 and IL-18 may be used to diagnose cardiovascular disease and qualitatively assess disease severity. Taken together, this data shows IL-17 and IL-18 are implicated in cardiovascular disease and provides a basis for treating cardiovascular disease by administering IL-17 and/or IL-18 antagonists, alone or in combination.

Example 2

Cytokine Profiles of Heart Transplant Recipients

[0280] This study shows, *inter alia*, that IL-17, IL-18 and soluble 4-1BB are elevated in heart transplant recipients that died within 72 hours of transplantation. This data shows that myocardial dysfunction is associated with significantly elevated levels of IL-17, IL-18 and soluble 4-1BB.

[0281] Cardiac transplantation remains a major therapeutic modality for patients with end-stage heart failure; however, the number of donor organs significantly limits its availability. This situation is worsened by the fact that around 20% of organ donors have such severe acute myocardial dysfunction associated with brain death that their hearts are unable to be used for transplantation (Hosenpud J D, et al., *Heart Lung Transplant*. 2001, 20(8):805-15). Brain death is a catastrophic event associated with marked activation of the immune system and elevated plasma levels of cytokines such as TNF α and IL6 (Takada, M, et al., *Transplantation* 1998, 65(12):1533-42 and Birks, E J, et al., *Transplant Proc*. 2001;33(5):2749-51). Cytokines are critical regulators of the T-helper 1 (Th1) and Th2 T-cell responses (Neurath, M F, et al., *Nat Med*. 2002, 8(6):567-73). The Th1 response results in pro-inflammatory cytokine release characterized by macrophage activation and, if unopposed, may result in tissue damage. The Th2 response results in a humoral immune response that in general opposes the Th1 response.

[0282] We hypothesized that death early after heart transplantation could be due to donor derived factors impacting on heart function either directly or indirectly, for example by initiating an acute rejection episode, ultimately resulting in myocardial dysfunction. To test this hypothesis we obtained plasma from two groups of heart transplant donors (as described above). Group A comprised samples from 16 organ donors where the recipient had an uneventful postoperative course and survived greater than 1 year. Group B samples were obtained from 14 donors where the recipients died within 72 hours of transplantation with myocardial dysfunction refractory to maximal medical therapy. The circulating levels of the Th1 (pro-inflammatory) cytokines interferon gamma (IFN γ), IL-12, IL-15, IL-17 and IL-18 along with the Th2 (anti-inflammatory) cytokines IL-4, IL-5, IL-10 and IL-13 were measured. Cytokine levels were determined using LUNIMEX® technology (Upstate, Waltham, Mass.), QUANTIKINE® ELISA kits (R&D Systems) or a custom made ELISA for soluble 4-1BB (using capture and detection antibodies, also from R&D systems). The control group consisted of 21 healthy patients with echocardiographically normal myocardial function undergoing routine coronary artery bypass graft surgery. The circulating Th2/Th1 ratio was obtained by dividing the sum of the Th2 cytokines by the sum of Th1 cytokines, both in pg/ml. In addition, as a potential marker of T-cell interaction with

antigen presenting cells, we also measured the level of the soluble receptor 4-1BB. 4-1BB is present on activated T-cells and 4-1BB ligand (4-1BB-L) is present on antigen presenting cells; engagement of 4-1BB by 4-1BB-L acts as a co-stimulatory signal (Kwon, B., et al., *Mol Cells*. 2000;10(2):119-26). Inflammatory mediator levels in groups A and B were compared to those in 21 non-brain dead control subjects.

[0283] **FIG. 9** shows that 4-1BB levels were significantly elevated in the patients with heart failure compared to normal subjects suggesting activation of this system in human heart failure, in common with rheumatoid arthritis patients (Eur J Immunol. 1998 January;28(1):290-5).

[0284] As shown in the Table 2, plasma samples from brain dead organ donors, where the recipients had an uneventful outcome post-transplantation (group A), contained significantly elevated levels of the Th2 cytokines IL-4, 10 and 13, along with the Th1 cytokines IFN γ , IL-12, 17 and 18 compared to control subjects. Overall, however, this maintained a circulating Th2/Th1 ratio of 0.32, which was not significantly different from 0.45 seen in control subjects. In group B, where the recipients died within 72 hours of transplantation, the Th2 cytokines IL-4 and 13 were unchanged from control levels, IL-10 was elevated compared to control subjects, but significantly reduced compared to Group A samples. IFN γ was elevated compared to control samples and IL-12 was unaltered. However, marked elevations in the levels of IL-17 and IL-18 were identified, resulting in a reduction in the Th2/Th1 ratio to 0.028 (P<0.05). Whilst soluble 4-1BB levels were similar in control and group A patients, significantly elevated levels were found in the plasma of group B patients (see **FIG. 4**).

[0285] These data identify a change in the circulating cytokine balance towards a pro-inflammatory (Th1) environment in the plasma of donors associated with early recipient death post-transplantation. Elevations in the levels of IL-17 and IL-18 are predominantly responsible for this. IL-18 utilizes the same signaling pathway as IL-1 and lipopolysaccharide (Sims, J E, *Curr Opin Immunol*. 2002;14(1):117-22), both of which are negatively inotropic. IL-17 induces nitric oxide production from a variety of cells, and also stimulates production of a number of cytokines and prostaglandins from a variety of cell types. The elevated levels of IL-17 and IL-18 seen, in particular, in group B patients could well contribute to the adverse recipient outcome early after transplantation. Significantly elevated levels of soluble 4-1BB were also observed in the plasma samples of donors in group B, suggesting that enhanced antigen presenting cell:T-cell interaction may occur in these patients.

[0286] Analysis of donor cytokine expression and levels of molecules such as IL-17, IL-18 and 4-1BB is valuable in identifying hearts from donors that may require intensive supportive therapy post-transplantation, or indeed that should not be used for transplantation. These data emphasize the importance of immune activation in brain death, its potential impact on outcome post-transplantation and the idea that therapies aimed at altering the donor cytokine balance may result in improved recipient outcome. These results suggest that elevated 4-1BB, as well as other cytokines such as IL-17 and IL-18, primes the heart for rejection in a recipient soon after transplantation or that these 4-1BB,

IL-17 and/or IL-18 may directly mediate impaired myocardial performance. Thus, the studies presented herein demonstrate that IL-17, IL-18 and 4-1BB are implicated in cardiovascular disease and provides a basis for treating cardiovascular disease by administering IL-17, IL-18 and/or 4-1BB antagonists, alone or in combination.

139:3630-3636 and Smith, S C, et al., *J. Immunol.* 1991, 147:2141-2147; in BALB/c mice: Pummarer, C L, et al., *J. Clin. Invest.* 1996, 97:2057-2062 and Liao, L, et al., *J. Clin. Invest.* 1993, 92:2877-2882; and in Lewis rats: Kodama, M, et al., *Clin. Immunol Immunopath.* 1991, 57:250-262 and Wegmann, K W, et al., *J. Immunol.* 1994, 153:892-900).

TABLE 2

	IL-4	IL-5	IL-10	IL-13	IFN γ	IL-12	IL-15	IL-17	IL-18	4-1BB
Control	2.6	0.9	1.7	1.9	1.9	10.4	38.7	1.1	68.1	30
mean (SE)	(0.9)	(0.3)	(0.3)	(0.7)	(0.4)	(4.7)	(13.85)	(0.5)	(23)	(20)
Group A	14	2.4	19.9	8.8	20.7	41.5	8.6	18	186	90
mean (SE)	(5.3)	(1.6)	(4.8)	(5.9)	(8.6)	(31.6)	(3.1)	(18)	(50)	(80)
Group B	3.5	0.45	7	1.1	8.4	7.4	5.4	292	373	420
mean (SE)	(0.4)	(0.1)	(2)	(0.2)	(1.5)	(1.9)	(1.8)	(70)	(51)	(170)

Example 3

IL-17 Levels and Heart Chamber Dimensions

[0287] These studies demonstrate that exposure to IL-17 results in a drop in left ventricle dimensions.

[0288] Female C57/Black6 mice were anesthetised with avertin. A midline laparotomy was performed and the inferior vena cava was carotidulated. A base line echocardiogram was then performed. Human IL-17 (200 ng) was then administered in a bolus of 200 ml of PBS (pH 7.4). At the indicated time points the echocardiogram was repeated and left ventricular internal diameter in diastole and systole were determined.

[0289] As shown in FIG. 4, these data demonstrate that IL-17, following acute administration, results in diminished chamber dimensions with maintained ejection fraction. The reduction in diastolic dimensions may suggest that IL-17 plays some part in mediating diastolic dysfunction, though it may also be involved in systolic heart failure. This data suggests IL-17 is implicated in cardiovascular disease and provides a basis for treating cardiovascular disease by administering IL-17 antagonists.

Example 4

IL-17 and IL-18 Levels are Elevated in a Murine Myosin-Induced Myocarditis Model

[0290] This study shows that IL-17 and IL-18 are elevated in an experimental autoimmune myocarditis (EAM) model, which shows a similar course of disease as seen in humans. The terms "EAM" and "cardiac myosin-induced myocarditis" are used interchangeably to describe similar models.

[0291] It is well known in the art that myocarditis is associated with an autoimmune process in which cardiac myosin is a major autoantigen. Cardiac myosin-induced myocarditis histologically resembles viral-induced myocarditis. It is generally agreed that both antibody and T-cells are implicated in inflammatory heart disease, such as myocarditis. Experimental autoimmune myocarditis models that mimic the disease in humans have been developed in a variety of rodent models and are well known in the art (see, for example, in A/J mice: Neu, N, et al., *J. Immunol.* 1987,

[0292] It has been shown in the A/J mouse EAM model that blocking IL-4 with anti-IL-4 monoclonal antibody reduced the severity of EAM by shifting the immune response from a Th2-like response to a Th1-like response with a concomitant increase in INF- γ production, which suggested INF- γ limits the disease. Blockade of INF- γ was shown to exacerbate disease, thereby establishing the basis for using an INF- γ knockout (INF- γ ^{-/-}) in a EAM model (Afanasyeva, M, et al., *Am J Pathol* 2001, 159:193-203).

[0293] Autoimmune myocarditis was induced in mice by immunizing BALB/c and INF- γ ^{-/-} knockout mice with 200 μ g α -myosin heavy chain plus MTB (mycobacterium tuberculosis) was included at 5 mg/ml H37Ra; Difco/Bectin Dickinson, Franklin Lakes, N.J.) and Pertussis toxin at 500 ng (List biological laboratories, Campbell, Calif.) intraperitoneally in a volume of 400 μ l. Interferon gamma-deficient mice were obtained from Jackson Laboratories, Bar Harbor, ME. BALB/c and INF- γ ^{-/-} were boosted on Day 7 by immunization with myosin formulated in Complete Freud's Adjuvant (CFA) with omission of Pertussis toxin. Animals were sacrificed at days 35, 55 or 85 and the following analysis were performed: histology, anti-myosin antibody titre, serum cytokine profile and antigen specific T-cell proliferation assays. Some animals underwent echocardiography.

[0294] BALB/c and INF- γ ^{-/-} mice that were immunized with α -myosin heavy chain developed antibodies against myosin peptide as measured by standard ELISA techniques. Myosin-immunized BALB/c and INF- γ ^{-/-} mice developed myocardial lesions, with the knockout mice showing an increase in lesion number and severity over negative controls and BALB/c mice. In addition, INF- γ knockout mice showed a greater degree of myocardial inflammation and fibrosis, as well as a greater percent increase in heart/body weight ratio over negative controls and BALB/c mice. Myosin-specific T-cell proliferation responses were shown in myosin-immunized INF- γ knockout and BALB/c mice, as measured by standard thymidine incorporation assays.

[0295] Furthermore, plasma levels of IL-17 and IL-18 were elevated in the cardiac myosin-induced myocarditis model. IL-17 and IL-18 were assayed using commercially available ELISA kits, such as described in Example 1. As shown in FIG. 5A, IL-17 levels were markedly increased in INF- γ ^{-/-} mice at day 28 and 35 post immunization. Plasma levels of IL-18 rose sharply at 9 days post immunization and

remained elevated out to day 28 (**FIG. 5B**). This data clearly shows that circulating plasma levels of IL-17 and IL-18 are elevated in the myosin-induced model and that IL-17 and IL-18 are associated with myocarditis immunopathology. This data provides a basis for treating cardiovascular disease by administering IL-17 and/or IL-18 antagonists, alone or in combination.

Example 5

IL-17 Expression in T-Cells in the Myosin-Induced Myocarditis Model

[0296] These experiments demonstrate that IL-17 is expressed at high levels in T-cell populations isolated from EAM mice. Details of the EAM model are provided in the previous Example.

[0297] T-cells were isolated from EAM mice using standard techniques and stimulated with anti-CD3 antibody. As shown in **FIG. 6A**, the animal immunized with cardiac myosin having histologically demonstrated cardiopathology (animal B) had significantly higher expression levels of IL-17 over negative controls (animals C and D), as well as an animal immunized with cardiac myosin, but not exhibiting signs of cardiopathology (animal A).

[0298] IL-17 expression in EAM mice was shown to be a myosin-specific T-cell response. On average, IL-17 levels were approximately 25 fold higher in T-cells isolated from animals immunized with cardiac myosin and exposed to antigen presenting cells fed myosin over antigen presenting cells not exposed to myosin (**FIG. 6B**).

[0299] In a related study, the antigen presenting cells were exposed to α -myosin peptide rather than being fed whole α -myosin protein. As in the previous study, T-cells from the animal immunized with cardiac myosin and having histological evidence of cardiopathology (animal B) released surprisingly high levels of IL-17 in response to antigen-specific stimulation by peptide-pulsed antigen presenting cells (**FIG. 6C**). Without being bound by theory, this study suggests that activated (likely CD4+) T-cells when encountering antigen presenting cells bearing heart antigens proliferate and release IL-17. The released IL-17 may contribute to inflammatory cell infiltration into the heart, direct myocardial damage or may also have a direct depressant effect on heart function.

Example 6

IL-18 and IL-18 Binding Protein Plasma Levels in Acute Coronary Syndrome Patients

[0300] These studies show that IL-18 is elevated in patients having acute coronary syndrome and that IL-18 correlates with increased risk for major adverse cardiac events (MACEs).

[0301] Patients were stratified into three patient groups: those having stable coronary artery disease (CAD), acute coronary syndrome (ACS) with cardiac troponin I (cTnI) plasma levels less than 0.4 ng/ml or acute coronary syndrome (ACS) with cardiac troponin I (cTnI) plasma levels greater than 0.4 ng/ml. The CAD group included patients having stable angina and the ACS group included patients

having unstable angina, non-ST elevation myocardial infarction, ST elevation myocardial infarction and sudden ischemic death.

[0302] Cardiac troponin I is recognized as a reliable biochemical marker for the diagnosis of myocardial injury, such as myocardial necrosis resulting from ischemia. Elevated cardiac troponin I is strongly associated with a high-risk profile for short and long term adverse cardiac events. Measuring the relative levels of cardiac troponin I provides a reliable stratification of risk and prediction of outcome for acute coronary syndrome patients. In this study, patients having a cardiac troponin I level of greater than 0.4 ng/ml suffered a serious adverse cardiac event, often resulting in death, within ten days.

[0303] Cytokine levels were measured in plasma samples from patients from each of the three groups essentially as described in the protocols provided in commercially available ELISA kits (see, for example, QUANTKINE® R&D Systems, Minneapolis, Minn., which provides assays for the quantitative determination of human IL-18). IL-18 Binding Protein A (IL-18 Bpa) was measured using commercially available antibodies from R&D. Plates were coated with a capture antibody. Samples were then added to wells and incubated at room temperature for 2 hours. Wells were then washed, incubated with a biotinylated detection antibody and the immunoreaction detected using standard techniques and TMB as chromogen. IL-18 Binding Protein produced in house was used as standard.

[0304] As shown in **FIG. 8A**, plasma levels of IL-18 was elevated in the ACS group having a cTnI level of less than 0.4 ng/ml, and more significantly, IL-18 levels were even higher in patients in the ACS group having a cTnI level of greater than 0.4 ng/ml. **FIG. 8B** shows the ratio of IL-18:IL-18 BPa among the three groups and illustrates that IL-18 Binding Protein levels are not elevated in either the ACS groups, which demonstrates that the elevated IL-18 levels are unopposed by this immune mechanism.

[0305] This data shows that circulating levels of IL-18 are elevated in ACS patients at high risk of subsequent major adverse cardiac events and that IL-18 Binding Protein levels do not rise concomitantly to counteract the immune response. Thus, IL-18 is implicated in cardiovascular disease and provides a basis for treating cardiovascular disease by administering IL-18 antagonists, alone or in combination with other antagonists described herein.

[0306] In addition, elevated IL-18 levels correlate with elevated cTnI levels and disease progression or disease severity. Therefore, IL-18 may also serve as a surrogate marker for increased risk for serious adverse cardiac events. Embodiments of the present invention include diagnostic assays for determining the level of IL-18 in patients having cardiovascular disease for the purpose of assessing disease progression or severity.

Example 7

4-1BB Ligand Knockout Mice are Protected in and Adriamycin®-Induced Murine Model of Dilated Cardiomyopathy

[0307] These experiments demonstrate that 4-1BB-L knockout mice (4-1BB-L^{-/-} or 4-1BB-L KO) showed no

mortality and had a delayed onset of cardiac dysfunction in an Adriamycin®-induced murine model of dilated cardiomyopathy.

[0308] Adriamycin® (Doxorubicin Hydrochloride, an anthracycline antibiotic-Pharmacia, Milan, Italy) has been shown to exhibit myocardial toxicity resulting in congestive heart failure, i.e., ischemic or dilated cardiomyopathy. Many animal models have been developed over the years using Adriamycin® and are well-known in the art.

[0309] Male 4-1BB-L^{-/-} (53 days old) and C57B1/6 (59 days old, Taconic, Germantown, N.Y.) were used in the study. Mice were anesthetized with 100-150 μ l ketamine-xylazine, weighed and ear tagged. Baseline echocardiographic (echo) measurements were taken from the parasternal long axis view while the mouse lay prone on a 1 cm thick agarose gel pad (SonosS500—Philips Co., with a S12 probe and a Instec heated microscope stage with 1 cm thick 1% agarose gelpad). Measurements taken included: AoR diameter, LA dimension, ACS, RVd, IVSd, LVIDd, LVPWd, IVSs, LVIDs, LVPWs, and HR. Echo evaluations were performed at weeks 0, 2.5, 5 and 7 and a phenotype assigned to each mouse based on the weekly echo measurements. Fractional shortening (FS) was calculated by the echo instrument. Measurements were normalized between mice by obtaining a PSLA image where the AoR and LA dimensions had a 1:1 ratio. Additionally, LV dimensions were taken from a Mmode image obtained by dissecting the LV perpendicularly through the PW, just distal to the tips of the mitral valve leaflets (sweep speed 100). Diastolic measurements were taken at the peak of the QRS complex of the EKG, while systolic measurements were recorded at the point of maximal contraction of the PW. All measurements were recorded onto optical disk, videotaped and printed.

[0310] After all echo measurements were obtained, the mice were injected retroorbitally with 22.5 mg/kg Adriamycin® (Sigma-Aldrich/Fluka, St. Louis, Mo.) and allowed to recover from anesthesia. Adriamycin was prepared from a powdered stock by dissolving 10 mg in 1 ml sterile water for a stock concentration of 10 mg/ml. Further dilutions were made with sterile saline. Solutions were made on the day of injection. The mice that received a dose of 22.5 mg/kg were injected with 150 μ l of a 3.75 mg/ml working dilution for a total dose of 0.5625 mg. Body weights were recorded daily.

[0311] If an animal was sacrificed due to a greater than 15% loss in body weight or at the end of the study, the hearts were harvested and fixed in 10% NBF (neutral buffered formalin). Briefly, thoracic incisions were made to expose the heart. Using a 27 g needle, 1M KCl (final concentration=50 mM) was injected directly into the heart to stop the hearts in diastole; PBS was then injected directly into the heart to flush blood out of the chambers. The heart was carefully removed, rinsed in PBS and placed into 10% NBF.

[0312] As mentioned above, a phenotype was assigned to each mouse based on the echo results and each mouse was classified according to the degree of cardiotoxicity into stages I, II, III or IV. The following criteria was used to phenotype the mice: Diastolic Dysfunction: LVIDd % Δ and LVIDs % Δ reduced >10% and FS same or increased; Systolic Dysfunction: LVIDd % Δ plus LVIDs % Δ increased >25% and FS reduced >12%; Diastolic and Systolic Dysfunction: LVIDd % Δ plus LVIDs % Δ reduced >20% and FS reduced >12%; Dilated: LVIDd % Δ >12% and LVIDs % Δ >12%.

[0313] 4 of 10 wild type mice died between 8 and 10 days after Adriamycin® injection. These deaths are considered chronic heart failure deaths. Wild-type mice challenged with Adriamycin® had an approximate 2-week mortality rate of 50% and approximately 66% of the mice exhibited evidence of cardiac dysfunction 2 to 2.5 weeks post Adriamycin®-challenge. The wild-type survivors generally showed two phenotypes: (a) systolic dysfunction with LV chamber dilation with diminished ejection fraction (EF), and (b) diastolic dysfunction with progressive reduction in chamber dimensions with diminished ventricular filling but maintained EF.

[0314] In contrast, no mortality was observed in the 4-1BB-L KO mice and none of the 4-1BB-L KO mice showed signs of the most severe cardiotoxicity following Adriamycin® challenge compared to 59 to 71% of wild type mice.

TABLE 3

	4-1BB-L KO	Wild Type B16	Historical B16 cntls
No Dysfunction (Class I)	7/14 (50%)	2/7 (29%)	9/22 (41%)
Transient Dysfunction (Class II)	1/14 (7%)	0/7 (0%)	1/22 (5%)
Progressive Dysfunction (Class III)	6/14 (43%)	1/7 (14%)	5/22 (23%)
Chronic Failure (Class IV)	0/14 (0%)	4/7 (57%)	7/22 (32%)
Total cardiotoxicity	7/14 (50%)	5/7 (71%)	13/22 (59%)

[0315] These results demonstrate that the 4-1BB and 4-1BB-L receptor:ligand pair are implicated in immune responses associated with cardiovascular disease, and in particular play a role in ischemic cardiomyopathy. These data establish a sound basis for preventing, treating or alleviating the symptoms of cardiovascular disease, and in particular ischemic cardiomyopathy, by antagonizing the interaction of 4-1BB and 4-1BB-L. Furthermore, these results demonstrate that antagonizing 4-1BB-L:4-1BB interactions reduces Adriamycin®-induced cardiotoxicity. Therefore, 4-1BB, CD30 and/or OX40 antagonists, may be used prior to or in combination with chemotherapeutic compositions in order to reduce the cardiotoxic side-effects of such therapeutics.

Example 8

4-1BB is Expressed by Damaged Cardiac Interstitial Cells and 4-1BB/4-1BBL

Signaling Contributes to Adriamycin®-Induced Cardiomyopathy in Mice

[0316] Co-stimulatory pathways have been implicated in myocarditis and dilated cardiomyopathy in mice and humans. Expression of co-stimulatory ligands is increased on cardiac myocytes, while the receptors are expressed on infiltrating immune/inflammatory cells (Seko et al, 2002; Seko et al, 2001; Seko et al, 1998). The aim of these studies was to determine the role of the 4-1BB/4-1BBL co-stimulatory pathway in the onset and progression of Adriamycin®-induced cardiomyopathy, which is not associated with the extensive inflammatory infiltrate seen with other forms

of dilated cardiomyopathy. These studies demonstrate a role for the 4-1BB/4-1BBL immune co-stimulatory pathway in Adriamycin®-induced cardiomyopathy, demonstrated a novel cardiac expression pattern of 4-1BB and implicated apoptosis as a mechanism of co-stimulatory contribution to Adriamycin®-induced cardiomyopathy.

[0317] 1. 4-1BB and 4-1BBL Expression is Up-Regulated in Adriamycin®-Treated Myocardium.

[0318] Six-week old C57B1/6 mice were injected retro-orbitally with 45 mg/kg Adriamycin® and the hearts were collected at 0, 24, 48, 72, and 96 hrs post treatment. Immunohistochemical staining on cryo-sections for CD45 was performed to detect inflammatory infiltrate, as well as staining for 4-1BB. Immunohistochemistry of CD45 did not recognize positive cells in Adriamycin®-treated myocardium. As positive control, CD45 positive cells were detected in spleen. While no inflammatory infiltrate was observed in the Adriamycin®-cardiomyopathy, we found expression of 4-1BB induced on 1-5% of cardiac interstitial cells within 2 days after Adriamycin® administration. As a percentage of animals demonstrating 4-1BB positive cells, 50% mice were positive for 4-1BB in myocardium at 48 hrs and 75% at 72 hrs. In immunohistochemical analysis, 4-1BBL increased in positivity after Adriamycin® treatment. Leukocyte counts in wild type and 4-1BBL-/- demonstrated similar level of neutrophils, monocytes and lymphocytes in peripheral blood, indicating the improvement of cardiac function by loss of 4-1BBL is unrelated to hematopoietic and inflammatory changes.

[0319] 2. Treatment with Agonistic Anti-4-1BB Antibody (M6) Accelerated Cardiac Dysfunction and m4-1BB-Fc Delayed and Reduced Cardiac Dysfunction.

[0320] An agonistic antibody to 4-1BB (M6) accelerated and exacerbated cardiac dysfunction in an Adriamycin® challenge model. In contrast, m4-1BB-Fc, which is a soluble decoy receptor for 4-1BBL, delayed and reduced cardiac dysfunction.

[0321] 100 ug/mouse of agonistic antibody to 4-1BB (M6, lot 9159-069 from Amgen Hybrodima Group) was administered IP on days 0, 3 and 6. Day 0 being the date of Adriamycin® challenge. NaCl controls were included for each treatment regimen. Wild type (WT), 4-1BBL KO, or WT mice treated with an activating antibody to 4-1BB (M6) were challenged with Adriamycin® in doses ranging from 22.5 to 45 mg/kg by retroorbital injection, immediately following baseline echocardiographic (ECHO) analysis of cardiac function. Serial ECHO analysis was performed at 1, 2.5 and/or 5 weeks after Adriamycin® challenge. In one study, WT and 4-1BBL KO mice were challenged with Adriamycin® (45 mg/kg) and hearts were collected at 24, 48 and 72 hours. TUNEL positivity by IHC and caspase 3 activation by western blot were determined as indices of apoptosis. 4-1BBL KO mice had improved cardiovascular function and decreased penetrance of cardiomyopathy, as evaluated by M-mode echocardiography compared to WT controls (see Table 4, below and FIG. 10). For Table 4, combination of stroke volume, systolic, diastolic end volume was used to categorize functional phenotype. 4-1BBL KO mice have reduced cardiotoxicity and improved function post-Adriamycin®. Treatment of WT C57B1/6 mice with the 4-1BB activating antibody (M6) accelerated the onset of ADR-induced cardiomyopathy (FIG. 11).

TABLE 4

Phenotype	WT	4-1BB-/- KO	M6 antibody	M4-1BB-Fc
Normal	48%	79%	53%	70%
Dysfunction	52%	21%	47%	30%

[0322] 3. Apoptosis was Increased in Adriamycin Myocardium.

[0323] C57B1/6 mice were treated with 45 mg/kg adriamycin. Heart tissues were harvested at different time points and analyzed for TUNEL positive nuclei. TUNEL positivity increased at 48 hrs and peaked at 72 hrs. Whole heart digests were collected from Adriamycin®-treated mice were stained with troponin-I(TnI) antibody and FITC conjugated secondary antibody and propidium iodide. TnI positive cells were analyzed for sub-G1 DNA fragments. In WT mice, cardiac TUNEL positivity increased at 48 and 72 hr after Adriamycin® injection.

[0324] Cardiac apoptosis, measured by TUNEL and sub-G1 DNA, was increased 3 days after Adriamycin®, concomitant with the increased expression of 4-1BB on interstitial cells. Chronic ongoing apoptosis, determined 5 weeks after Adriamycin® challenge when cardiac dysfunction is maximal in wild type but largely absent in 4-1BBL-/- mice, was lower in 4-1BBL-/- mice (1.5-fold vs baseline), compared to WT mice (4 fold). In a separate study, caspase 3 activation, determined by Western blot, was increased at 48 to 72 hrs post-Adriamycin® (45 mg/kg). In contrast, Adriamycin® did not induce caspase 3 cleavage in 4-1BBL-/- myocardium. Determined by western blot, Adriamycin® reduced phosphorylation of Akt in wild type but not 4-1BBL-/- hearts. Phosphorylation of JNK and p38 was not impacted by Adriamycin®. Therefore, 4-1BB/4-1BBL immune co-stimulatory pathway contributes to Adriamycin®-induced cardiomyopathy, possibly through modulation of Akt signaling to regulate

[0325] In conclusion, 4-1BBL deficient mice and 4-1BBL decoy receptor-treated mice conferred partial resistance to adriamycin induced cardiac damage, whereas 4-1BB activating antibody accelerated onset of damage, implying the contribution of 4-1BB to Adriamycin® effects in heart. Apoptosis, measured by TUNEL, sub-G1 DNA and activated caspase-3, was increased in Adriamycin®-treated wild type myocardium, but reduced in 4-1BBL-/. Phosphorylation of Akt was selectively suppressed by Adriamycin®, but maintained by loss of 4-1BBL, indicating the modulation of apoptosis by co-stimulatory pathway in heart is possibly through Akt, but not Jnk and p38 signaling. The consistency of decreased index of apoptosis and the improved cardiac function in 4-1BBL-/- suggests apoptosis play a pivotal role in Adriamycin®-induced cardiac deficiency.

Example 9

Antibodies Generated Against IL-17, IL-17R, IL-18, IL-18R, 4-1BB, 4-1BB-L, CD30, CD30-L and CD39

[0326] Monoclonal and/or polyclonal antibodies were generated against IL-17, IL-17R, IL-18, IL-18R, 4-1BB,

4-1BB-L, OX40, CD30, CD30-L and CD39 using standard techniques. One or more of these antibodies, or other antibodies directed against IL-17, IL-17R, IL-18, IL-18R, 4-1BB, 4-1BB-L, OX40, OX40-L, CD30, CD30-L or CD39 may be used as an antagonist for the treatment of cardiovascular disease. Immunogens used to generate antibodies included purified polypeptides, fragment thereof such as the extracellular domain, Fc-fusion proteins of the extracellular domains, and leucine-zipper derivatives of the extracellular domains (refer to Table 3 below). Of course, other forms of the proteins could be used to generate antibodies, such as any immunogenic fragment, alone or fused with other proteins. In addition, DNA encoding a polypeptide can be used as an immunogen, for example, DNA may be given intradermally (Raz et al., 1994, *Proc. Natl. Acad. Sci. USA* 91: 9519) or intamurally (Wang et al., 1993, *Proc. Natl. Acad. Sci. USA* 90: 4156); saline has been found to be a suitable diluent for DNA-based antigens, or by other similar techniques, as reviewed by Pardoll and Beckerleg in *Immunity* 3: 165, 1995.

[0327] In general, the antibodies were generated by the following method: rodents (BALB/c mice or Lewis rats, for example) were immunized with the polypeptide immunogen emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, M T)), and injected in amounts ranging from 10-100 micrograms subcutaneously or intraperitoneally. Ten days to three weeks days later, the immunized animals were boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

[0328] Serum samples were periodically taken by retro-orbital bleeding or tail-tip excision to test for polypeptide-specific antibodies by dot-blot assay, ELISA (enzyme-linked immunosorbent assay), immunoprecipitation, or other suitable assays, such as FACS analysis of antibody binding to its original immunogen. Following detection of an appropriate antibody titer, positive animals were provided one last intravenous injection of respective immunogen in saline. Three to four days later, the animals were sacrificed, spleen cells harvested and fused to a murine myeloma cell line, e.g., NS1 or preferably P3X63Ag8.653 (ATCC CRL-1580). The hybridoma cells were plated in multiple microtiter plates in a HAT (hypoxanthine, aminopterin and thymidine) selective medium to inhibit proliferation of non-fused cells, myeloma hybrids, and spleen cell hybrids.

[0329] Positive hybridoma cells were injected intraperitoneally into syngeneic rodents to produce ascites containing high concentrations of monoclonal antibodies. Alternatively, hybridoma cells were be grown in vitro in flasks or roller

bottles by various techniques. Monoclonal antibodies were purified by ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G has also been used.

[0330] Of course other conventional techniques may be used, such as those described in U.S. Pat. No. 4,411,993. For example, the immunogen preparation, choice of adjuvant and immunization protocol are well known in the art and may be found, for example in *Antibodies: A Laboratory Manual*, Harlow and Land (eds.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1988).

TABLE 4

Molecule	Ab name	Immunogen	Species	Type/Isotype
IL-17	mIL-17-M210	mCTLA8-Fc	Rat	mAb
IL-17	mIL-17-P1	mIL-17	Rabbit	polyclonal
IL-17R	hIL-17R-M202	hIL-17R-Fc (cos)	Mouse	IgG2a
IL-17R	hIL-17R-M203	hIL-17R-Fc (cos)	Mouse	IgG1
IL-17R	hIL-17R-M204	hIL-17R-Fc (cos)	Mouse	IgG2a
IL-17R	mIL-17R-M177	mIL-17R-Fc	Rat	IgG2a
IL-17R	mIL-17R-M178	mIL-17R-Fc	Rat	IgG2a
IL-17RH	mIL-17RH-M561	mIL-17RH-Fc	Rat	IgG2a
IL-17RH	mIL-17RH-M561	mIL-17RH-Fc	Rat	IgG2a
IL-18	hIL-1R/AcpL-P1	hIL-1R/AcpL-Fc	Rabbit	Polyclonal
IL-18	mIL-1R/AcpL-P1	mIL-1R/AcpL-Fc	Rabbit	Polyclonal
IL-18R	hIL-18R-M495	hIL-18R	Rat	IgG2b
IL-18R	hIL-18R-M496	hIL-18R	Rat	un-determined
IL-18R	hIL-18R-M497	hIL-18R	Rat	IgG2b
IL-18R	mIL-18R-M375	mRp1-Fc	Rat	IgG1
IL-18R	mIL-18R-M376	mIL-1R/RpL-Fc	Rat	IgG2a
IL-18R	mIL-18R-M377	mIL-1R/RpL-Fc	Rat	IgG1
IL-18R	hIL-18R-P1	h2F1 GST	Rabbit	Polyclonal
IL-18R	hIL-18R-P1	sol m2F1	Rabbit	Polyclonal
4-1BB	h41BB-M121	h4-1BB-Fc	Mouse	mAb/IgG1
4-1BB	h41BB-M127	h4-1BB-Fc	Mouse	mAb/IgG1
4-1BB	h41BB-M135	h4-1BB-Fc	Mouse	mAb/IgG1
4-1BB	h41BB-M4	sol h4-1BB-Fc	Mouse	mAb/IgM
4-1BB	h41BB-M8	sol h4-1BB-Fc	Mouse	mAb/IgG3
4-1BB	h41BB-M6	sol m4-1BB-Fc	Rat	mAb/IgG2a
4-1BB	m41BB-P1	sol m4-1BB-Fc	Rabbit	Polyclonal
4-1BB-L	m41BBL-M520	m4-1BBL-leuzip	Rat	mAb/IgG2a
4-1BB-L	m41BBL-P2	flag m4-1BBL	Rabbit	Polyclonal
CD30	hCD30-M44	hCD30-Fc	Mouse	mAb/IgG1
CD30	hCD30-M67	hCD30-Fc	Mouse	mAb/IgG1
CD30-L	hCD30L-M80	hCD30L-Fc/CV-1	Mouse	mAb/IgG2b
CD30-L	hCD30L-M81	hCD30L-Fc/CV-1	Mouse	mAb/IgG2b
CD30-L	hCD30L-M82	hCD30L(CHO)	Mouse	mAb/IgG2a
CD30-L	mCD30L-M115	mCD30L(CHO)	Rat	mAb/IgG2a
CD30-L	mCD30L-M30	mCD30L(CHO)	Rat	mAb/IgG2a
OX40	mOX40-M5	mOX40-Fc	Rat	IgG1
OX40	mOX40-M6	mOX40-Fc	Rat	IgG2b
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[0331]

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gcg ccg cgg tac ccc atg gac agg ttc gag gag gtg tac ttc cgc Ala Pro Arg Tyr Pro Leu Met Asp Arg Phe Glu Glu Val Tyr Phe Arg 520 525 530 535	1697
atc cag gac ctg gag atg ttc cag ccc ggc cgc atg cac ccc gta ggg Ile Gln Asp Leu Glu Met Phe Gln Pro Gly Arg Met His Arg Val Gly 540 545 550	1745
gag ctg tcg ggg gac aac tac ctg cgg agc ccc ggc ggc agg cag ctc Glu Leu Ser Gly Asp Asn Tyr Leu Arg Ser Pro Gly Arg Gln Leu 555 560 565	1793
cgc gcc ccc ctg gag agg ttc cgg gac tgg cag gtc cgc tgt ccc gac Arg Ala Ala Leu Asp Arg Phe Arg Asp Trp Gln Val Arg Cys Pro Asp 570 575 580	1841
tgg ttc gaa tgt gag aac ctc tac tca gca gat gac cag gat gcc ccc Trp Phe Glu Cys Glu Asn Leu Tyr Ser Ala Asp Asp Gln Asp Ala Pro 585 590 595	1889
tcc ctg gac gaa gag gtg ttt gag gac cca ctg ctg ccc ccc gga acc Ser Leu Asp Glu Glu Val Phe Glu Glu Pro Leu Leu Pro Pro Gly Thr 600 605 610 615	1937
ggc atc gtg aag cgg ggc ccc ctg gtg cgc gag cct ggc tcc cag gcc Gly Ile Val Lys Arg Ala Pro Leu Val Arg Glu Pro Gly Ser Gln Ala 620 625 630	1985

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tgc ctg gcc ata gac ccg ctg gtc ggg gag gaa gga gga gca gca gtc	2033
Cys Leu Ala Ile Asp Pro Leu Val Gly Glu Glu Gly Gly Ala Ala Val	
635 640 645	
gca aag ctg gaa cct cac ctg cag ccc cgg ggt cag cca gca gca cag	2081
Ala Lys Leu Glu Pro His Leu Gln Pro Arg Gly Gln Pro Ala Pro Gln	
650 655 660	
ccc ctc cac acc ctg gtg ctc gcc gca gag gag ggg gcc ctg gtg gcc	2129
Pro Leu His Thr Leu Val Leu Ala Ala Glu Glu Gly Ala Leu Val Ala	
665 670 675	
gcg gtg gag cct ggg ccc ctg gct gac ggt gcc gca gtc cgg ctg gca	2177
Ala Val Glu Pro Gly Pro Leu Ala Asp Gly Ala Ala Val Arg Leu Ala	
680 685 690 695	
ctg gcg ggg gag ggc gag gcc tgc ccg ctg ggc agc ccg ggc gct	2225
Leu Ala Gly Glu Ala Cys Pro Leu Leu Gly Ser Pro Gly Ala	
700 705 710	
ggg cga aat agc gtc ctc ttc ctc ccc gtg gac ccc gag gac tcg ccc	2273
Gly Arg Asn Ser Val Leu Phe Leu Pro Val Asp Pro Glu Asp Ser Pro	
715 720 725	
ctt ggc agc agc acc ccc atg gcg tct cct gac ctc ctt cca gag gac	2321
Leu Gly Ser Ser Thr Pro Met Ala Ser Pro Asp Leu Leu Pro Glu Asp	
730 735 740	
gtg agg gag cac ctc gaa ggc ttg atg ctc tcg ctc ttc gag cag agt	2369
Val Arg Glu His Leu Glu Gly Leu Met Leu Ser Leu Phe Glu Gln Ser	
745 750 755	
ctg agc tgc cag gcc cag ggg ggc tgc agt aga ccc gcc atg gtc ctc	2417
Leu Ser Cys Gln Ala Gln Gly Gly Cys Ser Arg Pro Ala Met Val Leu	
760 765 770 775	
aca gac cca cac acg ccc tac gag gag gag cag cgg cag tca gtg cag	2465
Thr Asp Pro His Thr Pro Tyr Glu Glu Gln Arg Gln Ser Val Gln	
780 785 790	
tct gac cag ggc tac atc tcc agg agc tcc ccg cag ccc gag gga	2513
Ser Asp Gln Gly Tyr Ile Ser Arg Ser Ser Pro Gln Pro Glu Gly	
795 800 805	
ctc acg gaa atg gag gaa gag gag gag cag gag cca ggg aag	2561
Leu Thr Glu Met Glu Glu Glu Glu Glu Gln Asp Pro Gly Lys	
810 815 820	
ccg gcc ctg cca ctc tct ccc gag gac ctg gag agc ctg agg agc ctc	2609
Pro Ala Leu Pro Leu Ser Pro Glu Asp Leu Glu Ser Leu Arg Ser Leu	
825 830 835	
cag cgg cag ctg ctt ttc cgc cag ctg cag aag aac tcg ggc tgg gac	2657
Gln Arg Gln Leu Leu Phe Arg Gln Leu Gln Lys Asn Ser Gly Trp Asp	
840 845 850 855	
acg atg ggg tca gag tca gag ggg ccc agt gca tga gggcggtcc	2703
Thr Met Gly Ser Glu Ser Gly Pro Ser Ala	
860 865	
ccagggaccg cccagatccc agctttgaga gaggagtgtg tgtgcacgta ttcatctgt	2763
tgtacatgtc tgcacatgtata tatgttcgtg tgtgaaatgt aggctttaaa atgtaaatgt	2823
ctggatTTTA atcccaggca tccctctaa cttttctttg tgcacggcgtc tggttatcg	2883
ctatccccag gggatccac acagccccgt cccaggagct aatggtagag cgtccctttag	2943
gctccattat tcgttcatttc agcatttattt gtgcacctac tatgtggcgg gcatTTGGGA	3003
taccaagata aattgcattgc ggcattggccc cagccatgaa ggaacttaac cgcttagtgcc	3063
gaggacacgt taaacgaaca ggtatggggccg ggcacgggtgg ctcacgcctgtc taatcccagc	3123
acactgggag gccgaggcag gtggatcaact ctgagggtcag gagtttgagc cagcctggcc	3183

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aacatggta aaccccgaa ttcgagctcg gtacccgggg	3223
<210> SEQ_ID NO 4	
<211> LENGTH: 866	
<212> TYPE: PRT	
<213> ORGANISM: Homo sapiens	
<400> SEQUENCE: 4	
Met Gly Ala Ala Arg Ser Pro Pro Ser Ala Val Pro Gly Pro Leu Leu	
1 5 10 15	
Gly Leu Leu Leu Leu Leu Gly Val Leu Ala Pro Gly Gly Ala Ser	
20 25 30	
Leu Arg Leu Leu Asp His Arg Ala Leu Val Cys Ser Gln Pro Gly Leu	
35 40 45	
Asn Cys Thr Val Lys Asn Ser Thr Cys Leu Asp Asp Ser Trp Ile His	
50 55 60	
Pro Arg Asn Leu Thr Pro Ser Ser Pro Lys Asp Leu Gln Ile Gln Leu	
65 70 75 80	
His Phe Ala His Thr Gln Gln Gly Asp Leu Phe Pro Val Ala His Ile	
85 90 95	
Glu Trp Thr Leu Gln Thr Asp Ala Ser Ile Leu Tyr Leu Glu Gly Ala	
100 105 110	
Glu Leu Ser Val Leu Gln Leu Asn Thr Asn Glu Arg Leu Cys Val Arg	
115 120 125	
Phe Glu Phe Leu Ser Lys Leu Arg His His His Arg Arg Trp Arg Phe	
130 135 140	
Thr Phe Ser His Phe Val Val Asp Pro Asp Gln Glu Tyr Glu Val Thr	
145 150 155 160	
Val His His Leu Pro Lys Pro Ile Pro Asp Gly Asp Pro Asn His Gln	
165 170 175	
Ser Lys Asn Phe Leu Val Pro Asp Cys Glu His Ala Arg Met Lys Val	
180 185 190	
Thr Thr Pro Cys Met Ser Ser Gly Ser Leu Trp Asp Pro Asn Ile Thr	
195 200 205	
Val Glu Thr Leu Glu Ala His Gln Leu Arg Val Ser Phe Thr Leu Trp	
210 215 220	
Asn Glu Ser Thr His Tyr Gln Ile Leu Leu Thr Ser Phe Pro His Met	
225 230 235 240	
Glu Asn His Ser Cys Phe Glu His Met His His Ile Pro Ala Pro Arg	
245 250 255	
Pro Glu Glu Phe His Gln Arg Ser Asn Val Thr Leu Thr Leu Arg Asn	
260 265 270	
Leu Lys Gly Cys Cys Arg His Gln Val Gln Ile Gln Pro Phe Phe Ser	
275 280 285	
Ser Cys Leu Asn Asp Cys Leu Arg His Ser Ala Thr Val Ser Cys Pro	
290 295 300	
Glu Met Pro Asp Thr Pro Glu Pro Ile Pro Asp Tyr Met Pro Leu Trp	
305 310 315 320	
Val Tyr Trp Phe Ile Thr Gly Ile Ser Ile Leu Leu Val Gly Ser Val	
325 330 335	
Ile Leu Leu Ile Val Cys Met Thr Trp Arg Leu Ala Gly Pro Gly Ser	
340 345 350	

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Glu Lys Tyr Ser Asp Asp Thr Lys Tyr Thr Asp Gly Leu Pro Ala Ala
 355 360 365
 Asp Leu Ile Pro Pro Pro Leu Lys Pro Arg Lys Val Trp Ile Ile Tyr
 370 375 380
 Ser Ala Asp His Pro Leu Tyr Val Asp Val Val Leu Lys Phe Ala Gln
 385 390 395 400
 Phe Leu Leu Thr Ala Cys Gly Thr Glu Val Ala Leu Asp Leu Leu Glu
 405 410 415
 Glu Gln Ala Ile Ser Glu Ala Gly Val Met Thr Trp Val Gly Arg Gln
 420 425 430
 Lys Gln Glu Met Val Glu Ser Asn Ser Lys Ile Ile Val Leu Cys Ser
 435 440 445
 Arg Gly Thr Arg Ala Lys Trp Gln Ala Leu Leu Gly Arg Gly Ala Pro
 450 455 460
 Val Arg Leu Arg Cys Asp His Gly Lys Pro Val Gly Asp Leu Phe Thr
 465 470 475 480
 Ala Ala Met Asn Met Ile Leu Pro Asp Phe Lys Arg Pro Ala Cys Phe
 485 490 495
 Gly Thr Tyr Val Val Cys Tyr Phe Ser Glu Val Ser Cys Asp Gly Asp
 500 505 510
 Val Pro Asp Leu Phe Gly Ala Ala Pro Arg Tyr Pro Leu Met Asp Arg
 515 520 525
 Phe Glu Glu Val Tyr Phe Arg Ile Gln Asp Leu Glu Met Phe Gln Pro
 530 535 540
 Gly Arg Met His Arg Val Gly Glu Leu Ser Gly Asp Asn Tyr Leu Arg
 545 550 555 560
 Ser Pro Gly Arg Gln Leu Arg Ala Ala Leu Asp Arg Phe Arg Asp
 565 570 575
 Trp Gln Val Arg Cys Pro Asp Trp Phe Glu Cys Glu Asn Leu Tyr Ser
 580 585 590
 Ala Asp Asp Gln Asp Ala Pro Ser Leu Asp Glu Glu Val Phe Glu Glu
 595 600 605
 Pro Leu Leu Pro Pro Gly Thr Gly Ile Val Lys Arg Ala Pro Leu Val
 610 615 620
 Arg Glu Pro Gly Ser Gln Ala Cys Leu Ala Ile Asp Pro Leu Val Gly
 625 630 635 640
 Glu Glu Gly Ala Ala Val Ala Lys Leu Glu Pro His Leu Gln Pro
 645 650 655
 Arg Gly Gln Pro Ala Pro Gln Pro Leu His Thr Leu Val Leu Ala Ala
 660 665 670
 Glu Glu Gly Ala Leu Val Ala Ala Val Glu Pro Gly Pro Leu Ala Asp
 675 680 685
 Gly Ala Ala Val Arg Leu Ala Leu Ala Gly Glu Gly Glu Ala Cys Pro
 690 695 700
 Leu Leu Gly Ser Pro Gly Ala Gly Arg Asn Ser Val Leu Phe Leu Pro
 705 710 715 720
 Val Asp Pro Glu Asp Ser Pro Leu Gly Ser Ser Thr Pro Met Ala Ser
 725 730 735
 Pro Asp Leu Leu Pro Glu Asp Val Arg Glu His Leu Glu Gly Leu Met
 740 745 750

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Leu Ser Leu Phe Glu Gln Ser Leu Ser Cys Gln Ala Gln Gly Gly Cys
 755 760 765
 Ser Arg Pro Ala Met Val Leu Thr Asp Pro His Thr Pro Tyr Glu Glu
 770 775 780
 Glu Gln Arg Gln Ser Val Gln Ser Asp Gln Gly Tyr Ile Ser Arg Ser
 785 790 795 800
 Ser Pro Gln Pro Pro Glu Gly Leu Thr Glu Met Glu Glu Glu Glu
 805 810 815
 Glu Glu Gln Asp Pro Gly Lys Pro Ala Leu Pro Leu Ser Pro Glu Asp
 820 825 830
 Leu Glu Ser Leu Arg Ser Leu Gln Arg Gln Leu Leu Phe Arg Gln Leu
 835 840 845
 Gln Lys Asn Ser Gly Trp Asp Thr Met Gly Ser Glu Ser Glu Gly Pro
 850 855 860
 Ser Ala
 865

<210> SEQ ID NO 5
 <211> LENGTH: 1626
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapeins
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1626)

 <400> SEQUENCE: 5

atg aat tgt aga gaa tta ccc ttg acc ctt tgg gtt ctt ata tct gta	48
Met Asn Cys Arg Glu Leu Pro Leu Thr Leu Trp Val Leu Ile Ser Val	
1 5 10 15	
agc act gca gaa tct tgt act tca cgt ccc cac att act gtt gtt gaa	96
Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu	
20 25 30	
ggg gaa cct ttc tat ctg aaa cat tgc tcg tgt tca ctt gca cat gag	144
Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu	
35 40 45	
att gaa aca acc acc aaa agc tgg tac aaa agc agt gga tca cag gaa	192
Ile Glu Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu	
50 55 60	
cat gtg gag ctg aac cca agg agt tcc tcg aga att gct ttg cat gat	240
His Val Glu Leu Asn Pro Arg Ser Ser Arg Ile Ala Leu His Asp	
65 70 75 80	
tgt gtt ttg gag ttt tgg cca gtt gag ttg aat gac aca gga tct tac	288
Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr	
85 90 95	
ttt ttc caa atg aaa aat tat act cag aaa tgg aaa tta aat gtc atc	336
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile	
100 105 110	
aga aga aat aaa cac agc tgt ttc act gaa aga caa gta act agt aaa	384
Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys	
115 120 125	
att gtg gaa gtt aaa aaa ttt ttg cag ata acc tgt gaa aac agt tac	432
Ile Val Glu Val Lys Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr	
130 135 140	
tat caa aca ctg gtc aac agc aca tca ttg tat aag aac tgt aaa aag	480
Tyr Gln Thr Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys	
145 150 155 160	
cta cta ctg gag aac aat aaa aac cca acg ata aag aac gcc gag	528

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Val	Glu	Arg	Lys	Ile	Lys	Ile	Ile	Leu	Ile	Glu	Phe	Thr	Pro	Val	Thr
465															480
gac ttc aca ttc ttg ccc caa tca cta aag ctt ttg aaa tct cac aga															1488
Asp	Phe	Thr	Phe	Leu	Pro	Gln	Ser	Leu	Lys	Leu	Leu	Lys	Ser	His	Arg
															495
485															
gtt ctg aag tgg aag gcc gat aaa tct ctt tct tat aac tca agg ttc															1536
Val	Leu	Lys	Trp	Lys	Ala	Asp	Lys	Ser	Leu	Ser	Tyr	Asn	Ser	Arg	Phe
															510
500															
tgg aag aac ctt ctt tac tta atg cct gca aaa aca gtc aag cca ggt															1584
Trp	Lys	Asn	Leu	Leu	Tyr	Leu	Met	Pro	Ala	Lys	Thr	Val	Lys	Pro	Gly
															525
515															
aga gac gaa ccg gaa gtc ttg cct gtt ctt tcc gag tct taa															1626
Arg	Asp	Glu	Pro	Glu	Val	Leu	Pro	Val	Leu	Ser	Glu	Ser			
															540
530															

<210> SEQ_ID NO 6

<211> LENGTH: 541

<212> TYPE: PRT

<213> ORGANISM: Homo sapeins

<400> SEQUENCE: 6

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1															15
Ser Thr Ala Glu Ser Cys Thr Ser Arg Pro His Ile Thr Val Val Glu															
															30
20															
Gly Glu Pro Phe Tyr Leu Lys His Cys Ser Cys Ser Leu Ala His Glu															
															45
35															
Ile Glu Thr Thr Lys Ser Trp Tyr Lys Ser Ser Gly Ser Gln Glu															
															60
50															
His Val Glu Leu Asn Pro Arg Ser Ser Ser Arg Ile Ala Leu His Asp															
65															80
Cys Val Leu Glu Phe Trp Pro Val Glu Leu Asn Asp Thr Gly Ser Tyr															
															95
85															
Phe Phe Gln Met Lys Asn Tyr Thr Gln Lys Trp Lys Leu Asn Val Ile															
															110
100															
Arg Arg Asn Lys His Ser Cys Phe Thr Glu Arg Gln Val Thr Ser Lys															
															125
115															
Ile Val Glu Val Lys Phe Phe Gln Ile Thr Cys Glu Asn Ser Tyr															
															140
130															
Tyr Gln Thr Leu Val Asn Ser Thr Ser Leu Tyr Lys Asn Cys Lys Lys															
145															160
Leu Leu Leu Glu Asn Asn Lys Asn Pro Thr Ile Lys Lys Asn Ala Glu															
															175
165															
Phe Glu Asp Gln Gly Tyr Tyr Ser Cys Val His Phe Leu His His Asn															
															190
180															
Gly Lys Leu Phe Asn Ile Thr Lys Thr Phe Asn Ile Thr Ile Val Glu															
															205
195															
Asp Arg Ser Asn Ile Val Pro Val Leu Leu Gly Pro Lys Leu Asn His															
															220
210															
Val Ala Val Glu Leu Gly Lys Asn Val Arg Leu Asn Cys Ser Ala Leu															
															240
225															
Leu Asn Glu Glu Asp Val Ile Tyr Trp Met Phe Gly Glu Glu Asn Gly															
															255
245															
Ser Asp Pro Asn Ile His Glu Glu Lys Glu Met Arg Ile Met Thr Pro															

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260	265	270	
Glu Gly Lys Trp His Ala Ser Lys Val Leu Arg Ile Glu Asn Ile Gly			
275	280	285	
Glu Ser Asn Leu Asn Val Leu Tyr Asn Cys Thr Val Ala Ser Thr Gly			
290	295	300	
Gly Thr Asp Thr Lys Ser Phe Ile Leu Val Arg Lys Ala Asp Met Ala			
305	310	315	320
Asp Ile Pro Gly His Val Phe Thr Arg Gly Met Ile Ile Ala Val Leu			
325	330	335	
Ile Leu Val Ala Val Val Cys Leu Val Thr Val Cys Val Ile Tyr Arg			
340	345	350	
Val Asp Leu Val Leu Phe Tyr Arg His Leu Thr Arg Arg Asp Glu Thr			
355	360	365	
Leu Thr Asp Gly Lys Thr Tyr Asp Ala Phe Val Ser Tyr Leu Lys Glu			
370	375	380	
Cys Arg Pro Glu Asn Gly Glu Glu His Thr Phe Ala Val Glu Ile Leu			
385	390	395	400
Pro Arg Val Leu Glu Lys His Phe Gly Tyr Lys Leu Cys Ile Phe Glu			
405	410	415	
Arg Asp Val Val Pro Gly Gly Ala Val Val Asp Glu Ile His Ser Leu			
420	425	430	
Ile Glu Lys Ser Arg Arg Leu Ile Ile Val Leu Ser Lys Ser Tyr Met			
435	440	445	
Ser Asn Glu Val Arg Tyr Glu Leu Glu Ser Gly Leu His Glu Ala Leu			
450	455	460	
Val Glu Arg Lys Ile Lys Ile Ile Leu Ile Glu Phe Thr Pro Val Thr			
465	470	475	480
Asp Phe Thr Phe Leu Pro Gln Ser Leu Lys Leu Leu Lys Ser His Arg			
485	490	495	
Val Leu Lys Trp Lys Ala Asp Lys Ser Leu Ser Tyr Asn Ser Arg Phe			
500	505	510	
Trp Lys Asn Leu Leu Tyr Leu Met Pro Ala Lys Thr Val Lys Pro Gly			
515	520	525	
Arg Asp Glu Pro Glu Val Leu Pro Val Leu Ser Glu Ser			
530	535	540	

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<210> SEQ_ID NO 7
<211> LENGTH: 2681
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CDS
<222> LOCATION: (484)..(2283)

<400> SEQUENCE: 7

ctctctggat aggaagaaaat atagtagaac cctttgaaaa tggatatttt cacatatttt 60
cgttcagata caaaaagctgg cagttactga aataaggact tgaagttcct tcctcttttt 120
ttatgtctta agagcagggaa ataaagagac agctgaaggt gtagccttga ccaactgaaa 180
gggaaatctt catcctctga aaaaacatata gtgattctca aaaaacgcac ctggaaaatt 240
gataaagaag cgattctgtta gattctccca gcgctgttgg gctctcaatt ccttctgtga 300
aggacaacat atggtgatgg gaaaaatcaga agctttgaga ccctctacac ctggatatga 360

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atcccccttc taatacttac cagaaatgaa gggatactc agggcagagt tctgaatctc	420
aaaacactct actctggcaa aggaatgaag ttattggagt gatgacagga acacggaga	480
aca atg ctc tgt ttg ggc tgg ata ttt ctt tgg ctt gtt gca gga gag Met Leu Cys Leu Gly Trp Ile Phe Leu Trp Leu Val Ala Gly Glu 1 5 10 15	528
cga att aaa gga ttt aat att tca ggt ttt tcc aca aaa aaa ctc ctt Arg Ile Lys Gly Phe Asn Ile Ser Gly Cys Ser Thr Lys Lys Leu Leu 20 25 30	576
tgg aca tat tct aca agg agt gaa gag gaa ttt gtc tta ttt tgt gat Trp Thr Tyr Ser Thr Arg Ser Glu Glu Glu Phe Val Leu Phe Cys Asp 35 40 45	624
tta cca gag cca cag aaa tca cat ttc tgc cac aga aat cga ctc tca Leu Pro Glu Pro Gln Lys Ser His Phe Cys His Arg Asn Arg Leu Ser 50 55 60	672
cca aaa caa gtc cct gag cac ctg ccc ttc atg ggt agt aac gac cta Pro Lys Gln Val Pro Glu His Leu Pro Phe Met Gly Ser Asn Asp Leu 65 70 75	720
tct gat gtc caa tgg tac caa caa cct tcg aat gga gat cca tta gag Ser Asp Val Gln Trp Tyr Gln Gln Pro Ser Asn Gly Asp Pro Leu Glu 80 85 90 95	768
gac att agg aaa agc tat cct cac atc att cag gac aaa tgt acc ctt Asp Ile Arg Lys Ser Tyr Pro His Ile Ile Gln Asp Lys Cys Thr Leu 100 105 110	816
cac ttt ttg acc cca ggg gtg aat aat tct ggg tca tat att tgt aga His Phe Leu Thr Pro Gly Val Asn Ser Gly Ser Tyr Ile Cys Arg 115 120 125	864
ccc aag atg att aag agc ccc tat gat gta gcc tgt tgt gtc aag atg Pro Lys Met Ile Lys Ser Pro Tyr Asp Val Ala Cys Cys Val Lys Met 130 135 140	912
att tta gaa gtt aag ccc cag aca aat gca tcc tgt gag tat tcc gca Ile Leu Glu Val Lys Pro Gln Thr Asn Ala Ser Cys Glu Tyr Ser Ala 145 150 155	960
tca cat aag caa gac cta ctt ctt ggg agc act ggc tct att tct tgc Ser His Lys Gln Asp Leu Leu Gly Ser Thr Gly Ser Ile Ser Cys 160 165 170 175	1008
ccc agt ctc agc tgc caa agt gat gca caa agt cca gcg gta acc tgg Pro Ser Leu Ser Cys Gln Ser Asp Ala Gln Ser Pro Ala Val Thr Trp 180 185 190	1056
tac aag aat gga aaa ctc ctc tct gtg gaa agg agc aac cga atc gta Tyr Lys Asn Gly Lys Leu Leu Ser Val Glu Arg Ser Asn Arg Ile Val 195 200 205	1104
gtg gat gaa gtt tat gac tat cac cag ggc aca tat gta tgt gat tac Val Asp Glu Val Tyr Asp Tyr His Gln Gly Thr Tyr Val Cys Asp Tyr 210 215 220	1152
act cag tcg gat act gtg agt tcg tgg aca gtc aga gct gtt gtt caa Thr Gln Ser Asp Thr Val Ser Ser Trp Thr Val Arg Ala Val Val Gln 225 230 235	1200
gtg aga acc att gtg gga gac act aaa ctc aaa cca gat att ctg gat Val Arg Thr Ile Val Gly Asp Thr Lys Leu Lys Pro Asp Ile Leu Asp 240 245 250 255	1248
cct gtc gag gac aca ctg gaa gta gaa ctt gga aag cct tta act att Pro Val Glu Asp Thr Leu Glu Val Glu Leu Gly Lys Pro Leu Thr Ile 260 265 270	1296
agc tgc aaa gca cga ttt ggc ttt gaa agg gtc ttt aac cct gtc ata Ser Cys Lys Ala Arg Phe Gly Phe Glu Arg Val Phe Asn Pro Val Ile 275 280 285	1344

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aaa tgg tac atc aaa gat tct gac cta gag tgg gaa gtc tca gta cct	1392
Lys Trp Tyr Ile Lys Asp Ser Asp Leu Glu Trp Glu Val Ser Val Pro	
290 295 300	
gag gcg aaa agt att aaa tcc act tta aag gat gaa atc att gag cgt	1440
Glu Ala Lys Ser Ile Lys Ser Thr Leu Lys Asp Glu Ile Ile Glu Arg	
305 310 315	
aat atc atc ttg gaa aaa gtc act cag cgt gat ctt cgc agg aag ttt	1488
Asn Ile Ile Leu Glu Lys Val Thr Gln Arg Asp Leu Arg Arg Lys Phe	
320 325 330 335	
gtt tgc ttt gtc cag aac tcc att gga aac aca acc cag tcc gtc caa	1536
Val Cys Phe Val Gln Asn Ser Ile Gly Asn Thr Thr Gln Ser Val Gln	
340 345 350	
ctg aaa gaa aag aga gga gtg gtc ctc ctg tac atc ctg ctt ggc acc	1584
Leu Lys Glu Lys Arg Gly Val Val Leu Leu Tyr Ile Leu Gly Thr	
355 360 365	
atc ggg acc ctg gtg gcc gtc ctg gcg gtc agt gcc ctc ctc tac agg	1632
Ile Gly Thr Leu Val Ala Val Leu Ala Ala Ser Ala Leu Leu Tyr Arg	
370 375 380	
cac tgg att gaa ata gtg ctg ctg tac cgg acc tac cag agc aag gat	1680
His Trp Ile Glu Ile Val Leu Leu Tyr Arg Thr Tyr Gln Ser Lys Asp	
385 390 395	
cag acg ctt ggg gat aaa aag gat ttt gat gct ttc gta tcc tat gca	1728
Gln Thr Leu Gly Asp Lys Lys Asp Phe Asp Ala Phe Val Ser Tyr Ala	
400 405 410 415	
aaa tgg agc tct ttt cca agt gag gcc act tca tct ctg agt gaa gaa	1776
Lys Trp Ser Ser Phe Pro Ser Glu Ala Thr Ser Ser Leu Ser Glu Glu	
420 425 430	
cac ttg gcc ctg agc cta ttt cct gat gtt tta gaa aac aaa tat gga	1824
His Leu Ala Leu Ser Leu Phe Pro Asp Val Leu Glu Asn Lys Tyr Gly	
435 440 445	
tat agc ctg tgt ttg ctt gaa aga gat gtg gct cca gga gga gtg tat	1872
Tyr Ser Leu Cys Leu Leu Glu Arg Asp Val Ala Pro Gly Gly Val Tyr	
450 455 460	
gca gaa gac att gtg agc att att aag aga agc aga aga gga ata ttt	1920
Ala Glu Asp Ile Val Ser Ile Ile Lys Arg Ser Arg Arg Gly Ile Phe	
465 470 475	
atc ttg agc ccc aac tat gtc aat gga ccc agt atc ttt gaa cta caa	1968
Ile Leu Ser Pro Asn Tyr Val Asn Gly Pro Ser Ile Phe Glu Leu Gln	
480 485 490 495	
gca gca gtg aat ctt gcc ttg gat gat caa aca ctg aaa ctc att tta	2016
Ala Ala Val Asn Leu Ala Leu Asp Asp Gln Thr Leu Lys Leu Ile Leu	
500 505 510	
att aag ttc tgt tac ttc caa gag cca gag tct cta cct cat ctc gtg	2064
Ile Lys Phe Cys Tyr Phe Gln Glu Pro Glu Ser Leu Pro His Leu Val	
515 520 525	
aaa aaa gct ctc agg gtt ttg ccc aca gtt act tgg aga ggc tta aaa	2112
Lys Lys Ala Leu Arg Val Leu Pro Thr Val Thr Trp Arg Gly Leu Lys	
530 535 540	
tca gtt cct ccc aat tct agg ttc tgg gcc aaa atg cgc tac cac atg	2160
Ser Val Pro Pro Asn Ser Arg Phe Trp Ala Lys Met Arg Tyr His Met	
545 550 555	
cct gtg aaa aac tct cag gga ttc acg tgg aac cag ctc aga att acc	2208
Pro Val Lys Asn Ser Gln Gly Phe Thr Trp Asn Gln Leu Arg Ile Thr	
560 565 570 575	
tct agg att ttt cag tgg aaa gga ctc agt aga aca gaa acc act ggg	2256
Ser Arg Ile Phe Gln Trp Lys Gly Leu Ser Arg Thr Glu Thr Thr Gly	
580 585 590	

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agg agc tcc cag cct aag gaa tgg tga aatgagccct ggagccccct	2303
Arg Ser Ser Gln Pro Lys Glu Trp	
595	
ccagtccagt ccctggata gagatgtgc tggacagaac tcacagctct gtgtgtgt	2363
gttcaggctg ataggaaatt caaagagtct cctgccagca ccaagcaagc ttgatggaca	2423
atggaatggg attgagactg tggtttagag cctttgattt cctggactgg acagacggcg	2483
agtgaattct ctagaccttgg ggtactttca gtacacaaca cccctaagat ttcccaagtgg	2543
tccgagcaga atcagaaaat acagctactt ctgccttatg gctagggAAC tgtcatgtct	2603
accatgtatt gtacatatga ctttatgtat acttgcaatc aaataaatat tattttatta	2663
gaaaaaaaaac cggaattc	2681

<210> SEQ ID NO 8

<211> LENGTH: 599

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Met Leu Cys Leu Gly Trp Ile Phe Leu Trp Leu Val Ala Gly Glu Arg	
1 5 10 15	
Ile Lys Gly Phe Asn Ile Ser Gly Cys Ser Thr Lys Lys Leu Leu Trp	
20 25 30	
Thr Tyr Ser Thr Arg Ser Glu Glu Phe Val Leu Phe Cys Asp Leu	
35 40 45	
Pro Glu Pro Gln Lys Ser His Phe Cys His Arg Asn Arg Leu Ser Pro	
50 55 60	
Lys Gln Val Pro Glu His Leu Pro Phe Met Gly Ser Asn Asp Leu Ser	
65 70 75 80	
Asp Val Gln Trp Tyr Gln Gln Pro Ser Asn Gly Asp Pro Leu Glu Asp	
85 90 95	
Ile Arg Lys Ser Tyr Pro His Ile Ile Gln Asp Lys Cys Thr Leu His	
100 105 110	
Phe Leu Thr Pro Gly Val Asn Asn Ser Gly Ser Tyr Ile Cys Arg Pro	
115 120 125	
Lys Met Ile Lys Ser Pro Tyr Asp Val Ala Cys Cys Val Lys Met Ile	
130 135 140	
Leu Glu Val Lys Pro Gln Thr Asn Ala Ser Cys Glu Tyr Ser Ala Ser	
145 150 155 160	
His Lys Gln Asp Leu Leu Gly Ser Thr Gly Ser Ile Ser Cys Pro	
165 170 175	
Ser Leu Ser Cys Gln Ser Asp Ala Gln Ser Pro Ala Val Thr Trp Tyr	
180 185 190	
Lys Asn Gly Lys Leu Leu Ser Val Glu Arg Ser Asn Arg Ile Val Val	
195 200 205	
Asp Glu Val Tyr Asp Tyr His Gln Gly Thr Tyr Val Cys Asp Tyr Thr	
210 215 220	
Gln Ser Asp Thr Val Ser Ser Trp Thr Val Arg Ala Val Val Gln Val	
225 230 235 240	
Arg Thr Ile Val Gly Asp Thr Lys Leu Lys Pro Asp Ile Leu Asp Pro	
245 250 255	
Val Glu Asp Thr Leu Glu Val Glu Leu Gly Lys Pro Leu Thr Ile Ser	
260 265 270	

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Cys Lys Ala Arg Phe Gly Phe Glu Arg Val Phe Asn Pro Val Ile Lys
 275 280 285
 Trp Tyr Ile Lys Asp Ser Asp Leu Glu Trp Glu Val Ser Val Pro Glu
 290 295 300
 Ala Lys Ser Ile Lys Ser Thr Leu Lys Asp Glu Ile Ile Glu Arg Asn
 305 310 315 320
 Ile Ile Leu Glu Lys Val Thr Gln Arg Asp Leu Arg Arg Lys Phe Val
 325 330 335
 Cys Phe Val Gln Asn Ser Ile Gly Asn Thr Thr Gln Ser Val Gln Leu
 340 345 350
 Lys Glu Lys Arg Gly Val Val Leu Leu Tyr Ile Leu Leu Gly Thr Ile
 355 360 365
 Gly Thr Leu Val Ala Val Leu Ala Ala Ser Ala Leu Leu Tyr Arg His
 370 375 380
 Trp Ile Glu Ile Val Leu Leu Tyr Arg Thr Tyr Gln Ser Lys Asp Gln
 385 390 395 400
 Thr Leu Gly Asp Lys Lys Asp Phe Asp Ala Phe Val Ser Tyr Ala Lys
 405 410 415
 Trp Ser Ser Phe Pro Ser Glu Ala Thr Ser Ser Leu Ser Glu Glu His
 420 425 430
 Leu Ala Leu Ser Leu Phe Pro Asp Val Leu Glu Asn Lys Tyr Gly Tyr
 435 440 445
 Ser Leu Cys Leu Leu Glu Arg Asp Val Ala Pro Gly Gly Val Tyr Ala
 450 455 460
 Glu Asp Ile Val Ser Ile Ile Lys Arg Ser Arg Arg Gly Ile Phe Ile
 465 470 475 480
 Leu Ser Pro Asn Tyr Val Asn Gly Pro Ser Ile Phe Glu Leu Gln Ala
 485 490 495
 Ala Val Asn Leu Ala Leu Asp Asp Gln Thr Leu Lys Leu Ile Leu Ile
 500 505 510
 Lys Phe Cys Tyr Phe Gln Glu Pro Glu Ser Leu Pro His Leu Val Lys
 515 520 525
 Lys Ala Leu Arg Val Leu Pro Thr Val Thr Trp Arg Gly Leu Lys Ser
 530 535 540
 Val Pro Pro Asn Ser Arg Phe Trp Ala Lys Met Arg Tyr His Met Pro
 545 550 555 560
 Val Lys Asn Ser Gln Gly Phe Thr Trp Asn Gln Leu Arg Ile Thr Ser
 565 570 575
 Arg Ile Phe Gln Trp Lys Gly Leu Ser Arg Thr Glu Thr Thr Gly Arg
 580 585 590
 Ser Ser Gln Pro Lys Glu Trp
 595

<210> SEQ ID NO 9
 <211> LENGTH: 644
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (66)..(599)

<400> SEQUENCE: 9

gagaagagga cgttgtcaca gataaagagc caggctcacc agtcctgac gcatgcata 60

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tgacc atg aga cac aac tgg aca cca gac ctc agc cct ttg tgg gtc ctg	110
Met Arg His Asn Trp Thr Pro Asp Leu Ser Pro Leu Trp Val Leu	
1 5 10 15	
ctc ctg tgt gcc cac gtc gtc act ctc ctg gtc aga gcc aca cct gtc	158
Leu Leu Cys Ala His Val Val Thr Leu Leu Val Arg Ala Thr Pro Val	
20 25 30	
tcg cag acc acc aca gct gcc act gcc tca gtt aga agc aca aag gac	206
Ser Gln Thr Thr Ala Ala Thr Ala Ser Val Arg Ser Thr Lys Asp	
35 40 45	
ccc tgc ccc tcc cag ccc cca gtg ttc cca gca gct aag cag cag tgt cca	254
Pro Cys Pro Ser Gln Pro Pro Val Phe Pro Ala Ala Lys Gln Cys Pro	
50 55 60	
gca ttg gaa gta acc tgg cca gag gta gaa gta cca ctg aat gga acg	302
Ala Leu Glu Val Thr Trp Pro Glu Val Glu Val Pro Leu Asn Gly Thr	
65 70 75	
ctg agc tta tcc tgt gta gcc tgc agc cgc ttc ccc aac ttc agc atc	350
Leu Ser Leu Ser Cys Val Ala Cys Ser Arg Phe Pro Asn Phe Ser Ile	
80 85 90 95	
ctc tac tgg ctg ggc aat ggt tcc ttc att gag cac ctc cca ggc cga	398
Leu Tyr Trp Leu Gly Asn Gly Ser Phe Ile Glu His Leu Pro Gly Arg	
100 105 110	
ctg tgg gag ggg agc acc agc cgg gaa cgt ggg agc aca ggt acg cag	446
Leu Trp Glu Gly Ser Thr Ser Arg Glu Arg Gly Ser Thr Gly Thr Gln	
115 120 125	
ctg tgc aag gcc ttg gta ctg gag cag ctg acc cct gcc ctg cac agc	494
Leu Cys Lys Ala Leu Val Leu Glu Gln Leu Thr Pro Ala Leu His Ser	
130 135 140	
acc aac ttc tcc tgt gta ctc gtc gac cct gaa cag gtt gtc cag cgt	542
Thr Asn Phe Ser Cys Val Leu Val Asp Pro Glu Gln Val Val Gln Arg	
145 150 155	
cac gtc gtc ctg gcc cag ctc tgg gct ggg ctg agg gca acc ttg ccc	590
His Val Val Leu Ala Gln Leu Trp Ala Gly Leu Arg Ala Thr Leu Pro	
160 165 170 175	
ccc acc caa gaagccctgc cctccagcca cagcagtcca cagcagcagg gttaa	644
Pro Thr Gln	

<210> SEQ ID NO 10

<211> LENGTH: 178

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

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

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Trp Glu Gly Ser Thr Ser Arg Glu Arg Gly Ser Thr Gly Thr Gln Leu
115 120 125

Cys Lys Ala Leu Val Leu Glu Gln Leu Thr Pro Ala Leu His Ser Thr
130 135 140

Asn Phe Ser Cys Val Leu Val Asp Pro Glu Gln Val Val Gln Arg His
145 150 155 160

Val Val Leu Ala Gln Leu Trp Ala Gly Leu Arg Ala Thr Leu Pro Pro
165 170 175

Thr Gln

<210> SEQ_ID NO 11
<211> LENGTH: 422
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

Met Arg His Asn Trp Thr Pro Asp Leu Ser Pro Leu Trp Val Leu Leu
1 5 10 15

Leu Cys Ala His Val Val Thr Leu Leu Val Arg Ala Thr Pro Val Ser
20 25 30

Gln Thr Thr Thr Ala Ala Thr Ala Ser Val Arg Ser Thr Lys Asp Pro
35 40 45

Cys Pro Ser Gln Pro Pro Val Phe Pro Ala Ala Lys Gln Cys Pro Ala
50 55 60

Leu Glu Val Thr Trp Pro Glu Val Glu Val Pro Leu Asn Gly Thr Leu
65 70 75 80

Ser Leu Ser Cys Val Ala Cys Ser Arg Phe Pro Asn Phe Ser Ile Leu
85 90 95

Tyr Trp Leu Gly Asn Gly Ser Phe Ile Glu His Leu Pro Gly Arg Leu
100 105 110

Trp Glu Gly Ser Thr Ser Arg Glu Arg Gly Ser Thr Gly Thr Gln Leu
115 120 125

Cys Lys Ala Leu Val Leu Glu Gln Leu Thr Pro Ala Leu His Ser Thr
130 135 140

Asn Phe Ser Cys Val Leu Val Asp Pro Glu Gln Val Val Gln Arg His
145 150 155 160

Val Val Leu Ala Gln Leu Trp Ala Gly Leu Arg Ala Thr Leu Pro Pro
165 170 175

Thr Gln Glu Ala Leu Pro Ser Ser His Ser Ser Pro Gln Gln Gly
180 185 190

Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
195 200 205

Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
210 215 220

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
225 230 235 240

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
245 250 255

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
260 265 270

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
275 280 285

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Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
 290 295 300

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
 305 310 315 320

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
 325 330 335

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
 340 345 350

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
 355 360 365

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
 370 375 380

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
 385 390 395 400

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
 405 410 415

Ser Leu Ser Pro Gly Lys
 420

<210> SEQ ID NO 12
 <211> LENGTH: 579
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(579)
 <220> FEATURE:
 <221> NAME/KEY: sig_peptide
 <222> LOCATION: (1)..(108)
 <220> FEATURE:
 <221> NAME/KEY: mat_peptide
 <222> LOCATION: (109)..()

<400> SEQUENCE: 12

atg gct gct gaa cca gta gaa gac aat tgc atc aac ttt gtg gca atg	48
Met Ala Ala Glu Pro Val Glu Asp Asn Cys Ile Asn Phe Val Ala Met	
-35 -30 -25	
aaa ttt att gac aat acg ctt tac ttt ata gct gaa gat gat gaa aac	96
Lys Phe Ile Asp Asn Thr Leu Tyr Phe Ile Ala Glu Asp Asp Glu Asn	
-20 -15 -10 -5	
ctg gaa tca gat tac ttt ggc aag ctt gaa tct aaa tta tca gtc ata	144
Leu Glu Ser Asp Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile	
-1 1 5 10	
aga aat ttg aat gac caa gtt ctc ttc att gac caa gga aat cgg cct	192
Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro	
15 20 25	
cta ttt gaa gat atg act gat tct gac tgt aga gat aat gca ccc cgg	240
Leu Phe Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg	
30 35 40	
acc ata ttt att ata agt atg tat aaa gat agc cag cct aga ggt atg	288
Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met	
45 50 55 60	
gct gta act atc tct gtg aag tgt gag aaa att tca ayt ctc tcc tgt	336
Ala Val Thr Ile Ser Val Lys Cys Glu Lys Ile Ser Xaa Leu Ser Cys	
65 70 75	
gag aac aaa att att tcc ttt aag gaa atg aat cct cct gat aac atc	384
Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile	
80 85 90	

-continued

aag gat aca aaa agt gac atc ata ttc ttt cag aga agt gtc cca gga	432
Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly	
95 100 105	
cat gat aat aag atg caa ttt gaa tct tca tca tac gaa gga tac ttt	480
His Asp Asn Lys Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe	
110 115 120	
cta gct tgt gaa aaa gag aga gac ctt ttt aaa ctc att ttg aaa aaa	528
Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys	
125 130 135 140	
gag gat gaa ttg ggg gat aga tct ata atg ttc act gtt caa aac gaa	576
Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu	
145 150 155	
gac	579
Asp	

<210> SEQ ID NO 13
 <211> LENGTH: 193
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: misc_feature
 <222> LOCATION: (73)..(73)
 <223> OTHER INFORMATION: The 'Xaa' at location 73 stands for Thr,
 or Ile.

<400> SEQUENCE: 13

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

<210> SEQ ID NO 14
 <211> LENGTH: 157
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 14

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn
 1 5 10 15

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp
 20 25 30

Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile
 35 40 45

Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
 50 55 60

Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile
 65 70 75 80

Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys
 85 90 95

Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys
 100 105 110

Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
 115 120 125

Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
 130 135 140

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
 145 150 155

<210> SEQ_ID NO 15

<211> LENGTH: 765

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (4)..(765)

<400> SEQUENCE: 15

gtc atg gaa tac gcc tct gac gct tca ctg gac ccc gaa gcc ccg tgg 48
 Met Glu Tyr Ala Ser Asp Ala Ser Leu Asp Pro Glu Ala Pro Trp
 1 5 10 15

cct ccc gcg ccc cgc gct cgc gcc tgc cgc gta ctg cct tgg gcc ctg 96
 Pro Pro Ala Pro Arg Ala Arg Ala Cys Arg Val Leu Pro Trp Ala Leu
 20 25 30

gtc gcg ggg ctg ctg ctg ctg ctc gct gcc gcc tgc gcc gtc 144
 Val Ala Gly Leu Leu Leu Leu Leu Leu Ala Ala Ala Cys Ala Val
 35 40 45

ttc ctc gcc tgc ccc tgg gcc gtg tcc ggg gct cgc gcc tgc ccc ggc 192
 Phe Leu Ala Cys Pro Trp Ala Val Ser Gly Ala Arg Ala Ser Pro Gly
 50 55 60

tcc gcg gcc agc ccg aga ctc cgc gag ggt ccc gag ctt tcg ccc gac 240
 Ser Ala Ala Ser Pro Arg Leu Arg Glu Gly Pro Glu Leu Ser Pro Asp
 65 70 75

gat ccc gcc ggc ctc ttg gac ctg cgg cag ggc atg ttt gcg cag ctg 288
 Asp Pro Ala Gly Leu Leu Asp Leu Arg Gln Gly Met Phe Ala Gln Leu
 80 85 90 95

gtg gcc caa aat gtt ctg ctg atc gat ggg ccc ctg agc tgg tac agt 336
 Val Ala Gln Asn Val Leu Leu Ile Asp Gly Pro Leu Ser Trp Tyr Ser
 100 105 110

gac cca ggc ctg gca ggc gtg tcc ctg acg ggg ggc ctg agc tac aaa 384
 Asp Pro Gly Leu Ala Gly Val Ser Leu Thr Gly Gly Leu Ser Tyr Lys
 115 120 125

-continued

gag gac acg aag gag ctg gtg gcc aag gct gga gtc tac tat gtc	432
Glu Asp Thr Lys Glu Leu Val Val Ala Lys Ala Gly Val Tyr Tyr Val	
130 135 140	
ttc ttt caa cta gag ctg cgg cgc gtg gtg gcc ggc gag ggc tca ggc	480
Phe Phe Gln Leu Leu Arg Arg Val Val Ala Gly Glu Gly Ser Gly	
145 150 155	
tcc gtt tca ctt gcg ctg cac ctg cag cca ctg cgc tct gct gct ggg	528
Ser Val Ser Leu Ala Leu His Leu Gln Pro Leu Arg Ser Ala Ala Gly	
160 165 170 175	
gcc gcc gcc ctg gct ttg acc gtg gac ctg cca ccc gcc tcc tcc gag	576
Ala Ala Ala Leu Ala Leu Thr Val Asp Leu Pro Pro Ala Ser Ser Glu	
180 185 190	
gct cgg aac tcg gcc ttc ggt ttc cag ggc cgc ttg ctg cac ctg agt	624
Ala Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser	
195 200 205	
gcc ggc cag cgc ctg ggc gtc cat ctt cac act gag gcc agg gca cgc	672
Ala Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg	
210 215 220	
cat gcc tgg cag ctt acc cag ggc gcc aca gtc ttg gga ctc ttc cgg	720
His Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg	
225 230 235	
gtg acc ccc gaa atc cca gcc gga ctc cct tca ccc agg tcg gaa	765
Val Thr Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser Glu	
240 245 250	

<210> SEQ ID NO 16

<211> LENGTH: 254

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

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

-continued

Arg Asn Ser Ala Phe Gly Phe Gln Gly Arg Leu Leu His Leu Ser Ala
 195 200 205

Gly Gln Arg Leu Gly Val His Leu His Thr Glu Ala Arg Ala Arg His
 210 215 220

Ala Trp Gln Leu Thr Gln Gly Ala Thr Val Leu Gly Leu Phe Arg Val
 225 230 235 240

Thr Pro Glu Ile Pro Ala Gly Leu Pro Ser Pro Arg Ser Glu
 245 250

<210> SEQ_ID NO 17
 <211> LENGTH: 1415
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (120)..(884)
 <220> FEATURE:
 <221> NAME/KEY: sig_peptide
 <222> LOCATION: (120)..(189)
 <220> FEATURE:
 <221> NAME/KEY: mat_peptide
 <222> LOCATION: (189)..()

<400> SEQUENCE: 17

agtggaaaagt tctccggcag ccctgagatc tcaagagtga catttgtgag accagcta 60
 ttgattaaaa ttctcttggaa atcagctttg ctagtatcat acctgtgccaa gatttcatc 119
 atg gga aac aac ttt tac aac ata gta gcc act ctg ttg ctg gtc ctc 167
 Met Gly Asn Ser Cys Tyr Asn Ile Val Ala Thr Leu Leu Val Leu
 -20 -15 -10

aac ttt gag agg aca aga tca ttg cag gat cct ttt agt aac tgc cca 215
 Asn Phe Glu Arg Thr Arg Ser Leu Gln Asp Pro Cys Ser Asn Cys Pro
 -5 -1 1 5

gct ggt aca ttc ttt gat aat aac agg aat cag att tgc agt ccc ttt 263
 Ala Gly Thr Phe Cys Asp Asn Asn Arg Asn Gln Ile Cys Ser Pro Cys
 10 15 20 25

cct cca aat aac ttc tcc agc gca ggt gga caa agg acc ttt gac ata 311
 Pro Pro Asn Ser Ser Ala Gly Gly Gln Arg Thr Cys Asp Ile
 30 35 40

tgc agg cag ttt aaa ggt gtt ttc agg acc agg aag gag ttt tcc tcc 359
 Cys Arg Gln Cys Lys Gly Val Phe Arg Thr Arg Lys Glu Cys Ser Ser
 45 50 55

acc agc aat gca gag ttt gac tgc act cca ggg ttt cac tgc ctg ggg 407
 Thr Ser Asn Ala Glu Cys Asp Cys Thr Pro Gly Phe His Cys Leu Gly
 60 65 70

gca gga tgc aac atg ttt gaa cag gat ttt aaa caa ggt caa gaa ctg 455
 Ala Gly Cys Ser Met Cys Glu Gln Asp Cys Lys Gln Gly Gln Glu Leu
 75 80 85

aca aaa aaa ggt ttt aaa gac ttt tgc ttt ggg aca ttt aac gat cag 503
 Thr Lys Lys Gly Cys Lys Asp Cys Cys Phe Gly Thr Phe Asn Asp Gln
 90 95 100 105

aaa cgt ggc atc ttt cga ccc tgg aca aac ttt tct ttt gat gga aag 551
 Lys Arg Gly Ile Cys Arg Pro Trp Thr Asn Cys Ser Leu Asp Gly Lys
 110 115 120

tct gtg ctt gtg aat ggg acg aag gag agg gac gtg gtc ttt gga cca 599
 Ser Val Leu Asn Gly Thr Lys Glu Arg Asp Val Val Cys Gly Pro
 125 130 135

tct cca gcc gac ctc tct ccg gga gca tcc tct gtg acc ccg cct gcc 647
 Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala
 140 145 150

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cct	gag	aga	gag	cca	gga	cac	tct	ccg	cag	atc	atc	tcc	ttc	ttt	ctt	695
Pro	Ala	Arg	Glu	Pro	Gly	His	Ser	Pro	Gln	Ile	Ile	Ser	Phe	Phe	Leu	
155				160				165								
gcg	ctg	acg	tcg	act	gcg	ttg	ctc	ctg	ctg	ttc	ttc	ctc	acg	ctc	743	
Ala	Leu	Thr	Ser	Thr	Ala	Leu	Leu	Phe	Leu	Leu	Phe	Phe	Leu	Thr	Leu	
170				175				180						185		
cgt	ttc	tct	gtt	gtt	aaa	cgg	ggc	aga	aag	aaa	ctc	ctg	tat	ata	ttc	791
Arg	Phe	Ser	Val	Val	Lys	Arg	Gly	Arg	Lys	Lys	Leu	Leu	Tyr	Ile	Phe	
190				195				200								
aaa	caa	cca	ttt	atg	aga	cca	gta	caa	act	act	caa	gag	gaa	gat	ggc	839
Lys	Gln	Pro	Phe	Met	Arg	Pro	Val	Gln	Thr	Thr	Gln	Glu	Glu	Asp	Gly	
205				210				215								
tgt	agc	tgc	cga	ttt	cca	gaa	gaa	gaa	gga	gga	tgt	gaa	ctg		884	
Cys	Ser	Cys	Arg	Phe	Pro	Glu	Glu	Glu	Gly	Gly	Cys	Glu	Leu			
220				225				230								
tgaaatggaa	gtcaataggg	ctgttggac	tttcttggaaa	agaagcaagg	aaatatgagt											944
catccgctat	cacagcttc	aaaagcaaga	acaccatcct	acataatacc	caggattccc											1004
ccaacacacg	ttcttttcta	aatgccaatg	agttggcctt	taaaaatgca	ccactttttt											1064
tttttttttt	gacagggtct	cactctgtca	cccaggctgg	agtgcagtg	caccaccatg											1124
gctctctgca	gccttgacct	ctgggagctc	aagtgtatcct	cctgcctcag	tctcctagta											1184
gctggaacta	caaggaaggg	ccaccacacc	tgactaactt	ttttgttttt	tttttgtttaa											1244
agatggcatt	tcgccatgtt	gtacaggctg	gtctcaaact	cctaggttca	ctttggcctc											1304
ccaaagtgtct	gggattacag	acatgaactg	ccaggccccgg	ccaaaataat	gcaccacttt											1364
taacagaaca	gacagatgag	gacagagctg	gtgataaaaa	aaaaaaaaaa	a											1415

<210> SEQ ID NO 18

<211> LENGTH: 255

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

Met	Gly	Asn	Ser	Cys	Tyr	Asn	Ile	Val	Ala	Thr	Leu	Leu	Leu	Val	Leu
-20						-15								-10	

Asn	Phe	Glu	Arg	Thr	Arg	Ser	Leu	Gln	Asp	Pro	Cys	Ser	Asn	Cys	Pro
-5						-1	1				5				

Ala	Gly	Thr	Phe	Cys	Asp	Asn	Asn	Arg	Asn	Gln	Ile	Cys	Ser	Pro	Cys
10						15			20			25			

Pro	Pro	Asn	Ser	Phe	Ser	Ser	Ala	Gly	Gly	Gln	Arg	Thr	Cys	Asp	Ile
30						35				40					

Cys	Arg	Gln	Cys	Lys	Gly	Val	Phe	Arg	Thr	Arg	Lys	Glu	Cys	Ser	Ser
45						50				55					

Thr	Ser	Asn	Ala	Glu	Cys	Asp	Cys	Thr	Pro	Gly	Phe	His	Cys	Leu	Gly
60						65				70					

Ala	Gly	Cys	Ser	Met	Cys	Glu	Gln	Asp	Cys	Lys	Gln	Gly	Gln	Glu	Leu
75						80				85					

Thr	Lys	Lys	Gly	Cys	Lys	Asp	Cys	Cys	Phe	Gly	Thr	Phe	Asn	Asp	Gln
90						95			100			105			

Lys	Arg	Gly	Ile	Cys	Arg	Pro	Trp	Thr	Asn	Cys	Ser	Leu	Asp	Gly	Lys
110						115			120						

Ser	Val	Leu	Val	Asn	Gly	Thr	Lys	Glu	Arg	Asp	Val	Val	Cys	Gly	Pro
125						130				135					

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Ser Pro Ala Asp Leu Ser Pro Gly Ala Ser Ser Val Thr Pro Pro Ala
 140 145 150
 Pro Ala Arg Glu Pro Gly His Ser Pro Gln Ile Ile Ser Phe Phe Leu
 155 160 165
 Ala Leu Thr Ser Thr Ala Leu Leu Phe Leu Leu Phe Phe Leu Thr Leu
 170 175 180 185
 Arg Phe Ser Val Val Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe
 190 195 200
 Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly
 205 210 215
 Cys Ser Cys Arg Phe Pro Glu Glu Glu Gly Cys Glu Leu
 220 225 230

<210> SEQ ID NO 19

<211> LENGTH: 648

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (1)..(645)

<400> SEQUENCE: 19

atg cat gtg ccg gcg ggc tcc gtg gcc agc cac ctg ggg acc acg agc	48
Met His Val Pro Ala Gly Ser Val Ala Ser His Leu Gly Thr Thr Ser	
1 5 10 15	
cgc agc tat ttc tat ttg acc aca gcc act ctg gct ctg tgc ctt gtc	96
Arg Ser Tyr Phe Tyr Leu Thr Thr Ala Thr Leu Ala Leu Cys Leu Val	
20 25 30	
ttc acg gtg gcc act att atg gtg ttg gtc gtt cag agg acg gac tcc	144
Phe Thr Val Ala Thr Ile Met Val Leu Val Val Gln Arg Thr Asp Ser	
35 40 45	
att ccc aac tca cct gac aac gtc ccc ctc aaa gga gga aat tgc tca	192
Ile Pro Asn Ser Pro Asp Asn Val Pro Leu Lys Gly Gly Asn Cys Ser	
50 55 60	
gaa gac ctc tta tgt atc ctg aaa aga gct cca ttc aag aag tca tgg	240
Glu Asp Leu Leu Cys Ile Leu Lys Arg Ala Pro Phe Lys Lys Ser Trp	
65 70 75 80	
gcc tac ctc caa gtg gca aag cat cta aac aaa acc aag ttg tct tgg	288
Ala Tyr Leu Gln Val Ala Lys His Leu Asn Lys Thr Lys Leu Ser Trp	
85 90 95	
aac aaa gat ggc att ctc cat gga gtc aga tat cag gat ggg aat ctg	336
Asn Lys Asp Gly Ile Leu His Gly Val Arg Tyr Gln Asp Gly Asn Leu	
100 105 110	
gtg atc caa ttc cct ggt ttg tac ttc atc att tgc caa ctg cag ttt	384
Val Ile Gln Phe Pro Gly Leu Tyr Phe Ile Ile Cys Gln Leu Gln Phe	
115 120 125	
ctt gta caa tgc cca aat aat tct gtc gat ctg aag ttg gag ctt ctc	432
Leu Val Gln Cys Pro Asn Asn Ser Val Asp Leu Lys Leu Glu Leu Leu	
130 135 140	
atc aac aag cat atc aaa aaa cag gcc ctg gtg aca gtg tgt gag tct	480
Ile Asn Lys His Ile Lys Lys Gln Ala Leu Val Thr Val Cys Glu Ser	
145 150 155 160	
gga atg caa acg aaa cac gta tac cag aat ctc tct caa ttc ttg ctg	528
Gly Met Gln Thr Lys His Val Tyr Gln Asn Leu Ser Gln Phe Leu Leu	
165 170 175	
gat tac ctg cag gtc aac acc acc ata tca gtc aat gtg gat aca ttc	576
Asp Tyr Leu Gln Val Asn Thr Thr Ile Ser Val Asn Val Asp Thr Phe	

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180	185	190	
cag tac ata gat aca agc acc ttt cct ctt gag aat gtg ttg tcc atc			624
Gln Tyr Ile Asp Thr Ser Thr Phe Pro Leu Glu Asn Val Leu Ser Ile			
195	200	205	

ttc tta tac agt aat tca gac tga			648
Phe Leu Tyr Ser Asn Ser Asp			
210	215		

<210> SEQ ID NO 20
 <211> LENGTH: 215
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 20

Met His Val Pro Ala Gly Ser Val Ala Ser His Leu Gly Thr Thr Ser			
1	5	10	15

Arg Ser Tyr Phe Tyr Leu Thr Thr Ala Thr Leu Ala Leu Cys Leu Val			
20	25	30	

Phe Thr Val Ala Thr Ile Met Val Leu Val Val Gln Arg Thr Asp Ser			
35	40	45	

Ile Pro Asn Ser Pro Asp Asn Val Pro Leu Lys Gly Gly Asn Cys Ser			
50	55	60	

Glu Asp Leu Leu Cys Ile Leu Lys Arg Ala Pro Phe Lys Lys Ser Trp			
65	70	75	80

Ala Tyr Leu Gln Val Ala Lys His Leu Asn Lys Thr Lys Leu Ser Trp			
85	90	95	

Asn Lys Asp Gly Ile Leu His Gly Val Arg Tyr Gln Asp Gly Asn Leu			
100	105	110	

Val Ile Gln Phe Pro Gly Leu Tyr Phe Ile Ile Cys Gln Leu Gln Phe			
115	120	125	

Leu Val Gln Cys Pro Asn Asn Ser Val Asp Leu Lys Leu Glu Leu Leu			
130	135	140	

Ile Asn Lys His Ile Lys Lys Gln Ala Leu Val Thr Val Cys Glu Ser			
145	150	155	160

Gly Met Gln Thr Lys His Val Tyr Gln Asn Leu Ser Gln Phe Leu Leu			
165	170	175	

Asp Tyr Leu Gln Val Asn Thr Thr Ile Ser Val Asn Val Asp Thr Phe			
180	185	190	

Gln Tyr Ile Asp Thr Ser Thr Phe Pro Leu Glu Asn Val Leu Ser Ile			
195	200	205	

Phe Leu Tyr Ser Asn Ser Asp			
210	215		

<210> SEQ ID NO 21
 <211> LENGTH: 705
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(702)

<400> SEQUENCE: 21

atg gac cca ggg ctg cag caa gca ctc aac gga atg gcc cct cct gga		48
Met Asp Pro Gly Leu Gln Gln Ala Leu Asn Gly Met Ala Pro Pro Gly		
1	5	10
		15

gac aca gcc atg cat gtg ccg gcg ggc tcc gtg gcc agc cac ctg ggg		96
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Asp Thr Ala Met His Val Pro Ala Gly Ser Val Ala Ser His Leu Gly	
20 25 30	
acc acg agc cgc agc tat ttc tat ttg acc aca gcc act ctg gct ctg	144
Thr Thr Ser Arg Ser Tyr Phe Tyr Leu Thr Thr Ala Thr Leu Ala Leu	
35 40 45	
tgc ctt gtc ttc acg gtg gcc act att atg gtg ttg gtc gtt cag agg	192
Cys Leu Val Phe Thr Val Ala Thr Ile Met Val Leu Val Val Gln Arg	
50 55 60	
acg gac tcc att ccc aac tca cct gac aac gtc ccc ctc aaa gga gga	240
Thr Asp Ser Ile Pro Asn Ser Pro Asp Asn Val Pro Leu Lys Gly Gly	
65 70 75 80	
aat tgc tca gaa gac ctc tta tgt atc ctg aaa aga gct cca ttc aag	288
Asn Cys Ser Glu Asp Leu Leu Cys Ile Leu Lys Arg Ala Pro Phe Lys	
85 90 95	
aag tca tgg gcc tac ctc caa gtg gca aag cat cta aac aaa acc aag	336
Lys Ser Trp Ala Tyr Leu Gln Val Ala Lys His Leu Asn Lys Thr Lys	
100 105 110	
ttg tct tgg aac aaa gat ggc att ctc cat gga gtc aga tat cag gat	384
Leu Ser Trp Asn Lys Asp Gly Ile Leu His Gly Val Arg Tyr Gln Asp	
115 120 125	
ggg aat ctg gtg atc caa ttc cct ggt ttg tac ttc atc att tgc caa	432
Gly Asn Leu Val Ile Gln Phe Pro Gly Leu Tyr Phe Ile Ile Cys Gln	
130 135 140	
ctg cag ttt ctt gta caa tgc cca aat aat tct gtc gat ctg aag ttg	480
Leu Gln Phe Leu Val Gln Cys Pro Asn Asn Ser Val Asp Leu Lys Leu	
145 150 155 160	
gag ctt ctc atc aac aag cat atc aaa aaa cag gcc ctg gtg aca gtg	528
Glu Leu Leu Ile Asn Lys His Ile Lys Lys Gln Ala Leu Val Thr Val	
165 170 175	
tgt gag tct gga atg caa acg aaa cac gta tac cag aat ctc tct caa	576
Cys Glu Ser Gly Met Gln Thr Lys His Val Tyr Gln Asn Leu Ser Gln	
180 185 190	
ttc ttg ctg gat tac ctg cag gtc aac acc acc ata tca gtc aat gtg	624
Phe Leu Leu Asp Tyr Leu Gln Val Asn Thr Thr Ile Ser Val Asn Val	
195 200 205	
gat aca ttc cag tac ata gat aca agc acc ttt cct ctt gag aat gtg	672
Asp Thr Phe Gln Tyr Ile Asp Thr Ser Thr Phe Pro Leu Glu Asn Val	
210 215 220	
ttg tcc atc ttc tta tac agt aat tca gac tga	705
Leu Ser Ile Phe Leu Tyr Ser Asn Ser Asp	
225 230	

<210> SEQ ID NO 22

<211> LENGTH: 234

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

Met Asp Pro Gly Leu Gln Gln Ala Leu Asn Gly Met Ala Pro Pro Gly	
1 5 10 15	

Asp Thr Ala Met His Val Pro Ala Gly Ser Val Ala Ser His Leu Gly	
20 25 30	

Thr Thr Ser Arg Ser Tyr Phe Tyr Leu Thr Thr Ala Thr Leu Ala Leu	
35 40 45	

Cys Leu Val Phe Thr Val Ala Thr Ile Met Val Leu Val Val Gln Arg	
50 55 60	

Thr Asp Ser Ile Pro Asn Ser Pro Asp Asn Val Pro Leu Lys Gly

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65	70	75	80
Asn Cys Ser Glu Asp Leu Leu Cys Ile Leu Lys Arg Ala Pro Phe Lys			
85	90	95	
Lys Ser Trp Ala Tyr Leu Gln Val Ala Lys His Leu Asn Lys Thr Lys			
100	105	110	
Leu Ser Trp Asn Lys Asp Gly Ile Leu His Gly Val Arg Tyr Gln Asp			
115	120	125	
Gly Asn Leu Val Ile Gln Phe Pro Gly Leu Tyr Phe Ile Ile Cys Gln			
130	135	140	
Leu Gln Phe Leu Val Gln Cys Pro Asn Asn Ser Val Asp Leu Lys Leu			
145	150	155	160
Glu Leu Leu Ile Asn Lys His Ile Lys Lys Gln Ala Leu Val Thr Val			
165	170	175	
Cys Glu Ser Gly Met Gln Thr Lys His Val Tyr Gln Asn Leu Ser Gln			
180	185	190	
Phe Leu Leu Asp Tyr Leu Gln Val Asn Thr Thr Ile Ser Val Asn Val			
195	200	205	
Asp Thr Phe Gln Tyr Ile Asp Thr Ser Thr Phe Pro Leu Glu Asn Val			
210	215	220	
Leu Ser Ile Phe Leu Tyr Ser Asn Ser Asp			
225	230		

<210> SEQ ID NO 23
 <211> LENGTH: 1788
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (1)..(1785)

<400> SEQUENCE: 23

atg cgc gtc ctc ctc gcc gcg ctg gga ctg ctg ttc ctg ggg gcg cta	48		
Met Arg Val Leu Leu Ala Ala Leu Gly Leu Leu Phe Leu Gly Ala Leu			
1	5	10	15
cga gcc ttc cca cag gat cga ccc ttc gag gac acc tgt cat gga aac	96		
Arg Ala Phe Pro Gln Asp Arg Pro Phe Glu Asp Thr Cys His Gly Asn			
20	25	30	
ccc agc cac tac tat gac aag gct gtc agg agg tgc tgt tac cgc tgc	144		
Pro Ser His Tyr Tyr Asp Lys Ala Val Arg Arg Cys Cys Tyr Arg Cys			
35	40	45	
ccc atg ggg ctg ttc ccg aca cag cag tgc cca cag agg cct act gac	192		
Pro Met Gly Leu Phe Pro Thr Gln Gln Cys Pro Gln Arg Pro Thr Asp			
50	55	60	
tgc agg aag cag tgt gag cct gac tac tac ctg gat gag gcc gac cgc	240		
Cys Arg Lys Gln Cys Glu Pro Asp Tyr Tyr Leu Asp Glu Ala Asp Arg			
65	70	75	80
tgt aca gcc tgc gtg act tgt tct cga gat gac ctc gtg gag aag acg	288		
Cys Thr Ala Cys Val Thr Cys Ser Arg Asp Asp Leu Val Glu Lys Thr			
85	90	95	
ccg tgt gca tgg aac tcc tcc cgt gtc tgc gaa tgt cga ccc ggc atg	336		
Pro Cys Ala Trp Asn Ser Ser Arg Val Cys Glu Cys Arg Pro Gly Met			
100	105	110	
ttc tgt tcc acg tct gcc aac tcc tgt gcc cgc tgc ttc ttc cat	384		
Phe Cys Ser Thr Ser Ala Val Asn Ser Cys Ala Arg Cys Phe Phe His			
115	120	125	
tct gtc tgt ccg gca ggg atg att gtc aag ttc cca ggc acg gcg cag	432		

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Ser Val Cys Pro Ala Gly Met Ile Val Lys Phe Pro Gly Thr Ala Gln		
130	135	140
aag aac acg gtc tgt gag ccg gct tcc cca ggg gtc agc cct gcc tgt	480	
Lys Asn Thr Val Cys Glu Pro Ala Ser Pro Gly Val Ser Pro Ala Cys		
145	150	155
160		
gcc agc cca gag aac tgc aag gaa ccc tcc agt ggc acc atc ccc cag	528	
Ala Ser Pro Glu Asn Cys Lys Glu Pro Ser Ser Gly Thr Ile Pro Gln		
165	170	175
gcc aag ccc acc ccg gtg tcc cca gca acc tcc agt gcc agc acc atg	576	
Ala Lys Pro Thr Pro Val Ser Pro Ala Thr Ser Ser Ala Ser Thr Met		
180	185	190
cct gta aga ggg ggc acc cgc ctc gcc cag gaa gct gct tct aaa ctg	624	
Pro Val Arg Gly Gly Thr Arg Leu Ala Gln Glu Ala Ala Ser Lys Leu		
195	200	205
acg agg gct ccc gac tct ccc tcc tct gtg gga agg cct agt tca gat	672	
Thr Arg Ala Pro Asp Ser Pro Ser Ser Val Gly Arg Pro Ser Ser Asp		
210	215	220
cca ggt ctg tcc cca aca cag cca tgc cca gag ggg tct ggt gat tgc	720	
Pro Gly Leu Ser Pro Thr Gln Pro Cys Pro Glu Gly Ser Gly Asp Cys		
225	230	235
240		
aga aag cag tgt gag ccc gac tac tac ctg gac gag gcc ggc cgc tgc	768	
Arg Lys Gln Cys Glu Pro Asp Tyr Tyr Leu Asp Glu Ala Gly Arg Cys		
245	250	255
aca gcc tgc gtg agc tgt tct cga gat gac ctt gtg gag aag acg cca	816	
Thr Ala Cys Val Ser Cys Ser Arg Asp Asp Leu Val Glu Lys Thr Pro		
260	265	270
tgt gca tgg aac tcc tcc cgc acc tgc gaa tgt cga cct ggc atg atc	864	
Cys Ala Trp Asn Ser Ser Arg Thr Cys Glu Cys Arg Pro Gly Met Ile		
275	280	285
tgt gcc aca tca gcc acc aac tcc tgt gcc cgc tgt gtc ccc tac cca	912	
Cys Ala Thr Ser Ala Thr Asn Ser Cys Ala Arg Cys Val Pro Tyr Pro		
290	295	300
atc tgt gca gga gag acg gtc acc aag ccc cag gat atg gct gag aag	960	
Ile Cys Ala Gly Glu Thr Val Thr Lys Pro Gln Asp Met Ala Glu Lys		
305	310	315
320		
gac acc acc ttt gag ggc cca ccc ctg ggg acc cag ccg gac tgc aac	1008	
Asp Thr Thr Phe Glu Ala Pro Pro Leu Gly Thr Gln Pro Asp Cys Asn		
325	330	335
ccc acc cca gag aat ggc gag ggc cct gcc agc acc agc ccc act cag	1056	
Pro Thr Pro Glu Asn Gly Glu Ala Pro Ala Ser Thr Ser Pro Thr Gln		
340	345	350
agc ttg ctg gtg gac tcc cag gcc agt aag acg ctg ccc atc cca acc	1104	
Ser Leu Leu Val Asp Ser Gln Ala Ser Lys Thr Leu Pro Ile Pro Thr		
355	360	365
agc gct ccc gtc gct ctc tcc acg ggg aag ccc gtt ctg gat gca	1152	
Ser Ala Pro Val Ala Leu Ser Ser Thr Gly Lys Pro Val Leu Asp Ala		
370	375	380
ggg cca gtg ctc ttc tgg gtg atc ctg gtg ttg gtt gtg gtg gtc ggc	1200	
Gly Pro Val Leu Phe Trp Val Ile Leu Val Leu Val Val Val Gly		
385	390	395
400		
tcc agc gcc ttc ctc ctg tgc cac cgg agg gcc tgc agg aag cga att	1248	
Ser Ser Ala Phe Leu Leu Cys His Arg Arg Ala Cys Arg Lys Arg Ile		
405	410	415
cgg cag aag ctc cac ctg tgc tac ccg gtc cag acc tcc cag ccc aag	1296	
Arg Gln Lys Leu His Leu Cys Tyr Pro Val Gln Thr Ser Gln Pro Lys		
420	425	430
cta gag ctt gtg gat tcc aga ccc agg agg agc tca acg cag ctg agg	1344	

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Leu	Glu	Lue	Val	Asp	Ser	Arg	Pro	Arg	Arg	Ser	Ser	Thr	Gln	Leu	Arg
435							440						445		
agt ggt gcg tcg gtg aca gaa ccc gtc gcg gaa gag cga ggg tta atg															1392
Ser	Gly	Ala	Ser	Val	Thr	Glu	Pro	Val	Ala	Glu	Glu	Arg	Gly	Leu	Met
450							455						460		
agc cag cca ctg atg gag acc tgc cac agc gtg ggg gca gcc tac ctg															1440
Ser	Gln	Pro	Leu	Met	Glu	Thr	Cys	His	Ser	Val	Gly	Ala	Ala	Tyr	Leu
465							470						475		
gag agc ctg ccg ctg cag gat gcc agc ccg gcc ggg ggc ccc tcg tcc															1488
Glu	Ser	Leu	Pro	Leu	Gln	Asp	Ala	Ser	Pro	Ala	Gly	Gly	Pro	Ser	Ser
485							490						495		
ccc agg gag ctt cct gag ccc ccg gtg tcc acg gag cac acc aat aac															1536
Pro	Arg	Asp	Leu	Pro	Glu	Pro	Arg	Val	Ser	Thr	Glu	His	Thr	Asn	Asn
500							505						510		
aag att gag aaa atc tac atc atg aag gct gac acc gtg atc gtg ggg															1584
Lys	Ile	Glu	Lys	Ile	Tyr	Ile	Met	Lys	Ala	Asp	Thr	Val	Ile	Val	Gly
515							520						525		
acc gtg aag gct gag ctg ccg gag ggc cgg ggc ctg gcg ggg cca gca															1632
Thr	Val	Lys	Ala	Glu	Leu	Pro	Glu	Gly	Arg	Gly	Leu	Ala	Gly	Pro	Ala
530							535						540		
gag ccc gag ttg gag gag ctg gag ggc gac cat acc ccc cac tac															1680
Glu	Pro	Glu	Leu	Glu	Glu	Leu	Glu	Ala	Asp	His	Thr	Pro	His	Tyr	
545							550						555		
560															
ccc gag cag gag aca gaa ccg cct ctg ggc agc tgc agc gat gtc atg															1728
Pro	Glu	Gln	Glu	Thr	Glu	Pro	Pro	Leu	Gly	Ser	Cys	Ser	Asp	Val	Met
565							570						575		
ctc tca gtg gaa gag gaa ggg aaa gaa gac ccc ttg ccc aca gct gcc															1776
Leu	Ser	Val	Glu	Glu	Glu	Gly	Lys	Glu	Asp	Pro	Leu	Pro	Thr	Ala	Ala
580							585						590		
tct gga aag tga															1788
Ser	Gly	Lys													
595															
<210> SEQ ID NO 24															
<211> LENGTH: 595															
<212> TYPE: PRT															
<213> ORGANISM: Homo sapiens															
<400> SEQUENCE: 24															
Met	Arg	Val	Leu	Leu	Ala	Ala	Leu	Gly	Leu	Leu	Phe	Leu	Gly	Ala	Leu
1							5					10			15
Arg Ala Phe Pro Gln Asp Arg Pro Phe Glu Asp Thr Cys His Gly Asn															
20							25					30			
Pro Ser His Tyr Tyr Asp Lys Ala Val Arg Arg Cys Cys Tyr Arg Cys															
35							40					45			
Pro Met Gly Leu Phe Pro Thr Gln Gln Cys Pro Gln Arg Pro Thr Asp															
50							55					60			
Cys Arg Lys Gln Cys Glu Pro Asp Tyr Tyr Leu Asp Glu Ala Asp Arg															
65							70					75			80
Cys Thr Ala Cys Val Thr Cys Ser Arg Asp Asp Leu Val Glu Lys Thr															
85							90					95			
Pro Cys Ala Trp Asn Ser Ser Arg Val Cys Glu Cys Arg Pro Gly Met															
100							105					110			
Phe Cys Ser Thr Ser Ala Val Asn Ser Cys Ala Arg Cys Phe Phe His															
115							120					125			
Ser Val Cys Pro Ala Gly Met Ile Val Lys Phe Pro Gly Thr Ala Gln															

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130	135	140
Lys Asn Thr Val Cys Glu Pro Ala Ser Pro Gly Val Ser Pro Ala Cys		
145	150	155
Ala Ser Pro Glu Asn Cys Lys Glu Pro Ser Ser Gly Thr Ile Pro Gln		
165	170	175
Ala Lys Pro Thr Pro Val Ser Pro Ala Thr Ser Ser Ala Ser Thr Met		
180	185	190
Pro Val Arg Gly Gly Thr Arg Leu Ala Gln Glu Ala Ala Ser Lys Leu		
195	200	205
Thr Arg Ala Pro Asp Ser Pro Ser Ser Val Gly Arg Pro Ser Ser Asp		
210	215	220
Pro Gly Leu Ser Pro Thr Gln Pro Cys Pro Glu Gly Ser Gly Asp Cys		
225	230	235
Arg Lys Gln Cys Glu Pro Asp Tyr Tyr Leu Asp Glu Ala Gly Arg Cys		
245	250	255
Thr Ala Cys Val Ser Cys Ser Arg Asp Asp Leu Val Glu Lys Thr Pro		
260	265	270
Cys Ala Trp Asn Ser Ser Arg Thr Cys Glu Cys Arg Pro Gly Met Ile		
275	280	285
Cys Ala Thr Ser Ala Thr Asn Ser Cys Ala Arg Cys Val Pro Tyr Pro		
290	295	300
Ile Cys Ala Gly Glu Thr Val Thr Lys Pro Gln Asp Met Ala Glu Lys		
305	310	315
Asp Thr Thr Phe Glu Ala Pro Pro Leu Gly Thr Gln Pro Asp Cys Asn		
325	330	335
Pro Thr Pro Glu Asn Gly Glu Ala Pro Ala Ser Thr Ser Pro Thr Gln		
340	345	350
Ser Leu Leu Val Asp Ser Gln Ala Ser Lys Thr Leu Pro Ile Pro Thr		
355	360	365
Ser Ala Pro Val Ala Leu Ser Ser Thr Gly Lys Pro Val Leu Asp Ala		
370	375	380
Gly Pro Val Leu Phe Trp Val Ile Leu Val Leu Val Val Val Gly		
385	390	395
Ser Ser Ala Phe Leu Leu Cys His Arg Arg Ala Cys Arg Lys Arg Ile		
405	410	415
Arg Gln Lys Leu His Leu Cys Tyr Pro Val Gln Thr Ser Gln Pro Lys		
420	425	430
Leu Glu Leu Val Asp Ser Arg Pro Arg Arg Ser Ser Thr Gln Leu Arg		
435	440	445
Ser Gly Ala Ser Val Thr Glu Pro Val Ala Glu Glu Arg Gly Leu Met		
450	455	460
Ser Gln Pro Leu Met Glu Thr Cys His Ser Val Gly Ala Ala Tyr Leu		
465	470	475
Glu Ser Leu Pro Leu Gln Asp Ala Ser Pro Ala Gly Gly Pro Ser Ser		
485	490	495
Pro Arg Asp Leu Pro Glu Pro Arg Val Ser Thr Glu His Thr Asn Asn		
500	505	510
Lys Ile Glu Lys Ile Tyr Ile Met Lys Ala Asp Thr Val Ile Val Gly		
515	520	525
Thr Val Lys Ala Glu Leu Pro Glu Gly Arg Gly Leu Ala Gly Pro Ala		
530	535	540

-continued

Glu Pro Glu Leu Glu Glu Leu Glu Ala Asp His Thr Pro His Tyr
 545 550 555 560

Pro Glu Gln Glu Thr Glu Pro Pro Leu Gly Ser Cys Ser Asp Val Met
 565 570 575

Leu Ser Val Glu Glu Gly Lys Glu Asp Pro Leu Pro Thr Ala Ala
 580 585 590

Ser Gly Lys
 595

<210> SEQ ID NO 25

<211> LENGTH: 696

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<221> NAME/KEY: CDS

<222> LOCATION: (138)..(686)

<400> SEQUENCE: 25

ggccctggga ccttgccta tttctgatt gataggctt gtttgtctt taccccttc 60

tttctgggaa aaacctcagt ttatcgac gttcccttt tccatatctt catctccct 120

ctacccagat tgtgaag atg gaa agg gtc caa ccc ctg gaa gag aat gtg 170
 Met Glu Arg Val Gln Pro Leu Glu Glu Asn Val
 1 5 10

gga aat gca gcc agg cca aga ttc gag agg aac aag cta ttg ctg gtg 218
 Gly Asn Ala Arg Pro Arg Phe Glu Arg Asn Lys Leu Leu Val
 15 20 25

gcc tct gta att cag gga ctg ggg ctg ctc ctg tgc ttc acc tac atc 266
 Ala Ser Val Ile Gln Gly Leu Gly Leu Leu Leu Cys Phe Thr Tyr Ile
 30 35 40

tgc ctg cac ttc tct gct ctt cag gta tca cat cgg tat cct cga att 314
 Cys Leu His Phe Ser Ala Leu Gln Val Ser His Arg Tyr Pro Arg Ile
 45 50 55

caa agt atc aaa gta caa ttt acc gaa tat aag aag gag aaa ggt ttc 362
 Gln Ser Ile Lys Val Phe Thr Glu Tyr Lys Lys Glu Lys Gly Phe
 60 65 70 75

atc ctc act tcc caa aag gag gat gaa atc atg aag gtg cag aac aac 410
 Ile Leu Thr Ser Gln Lys Glu Asp Glu Ile Met Lys Val Gln Asn Asn
 80 85 90

tca gtc atc atc aac tgt gat ggg ttt tat ctc atc tcc ctg aag ggc 458
 Ser Val Ile Ile Asn Cys Asp Gly Phe Tyr Leu Ile Ser Leu Lys Gly
 95 100 105

tac ttc tcc cag gaa gtc aac att agc ctt cat tac cag aag gat gag 506
 Tyr Phe Ser Gln Glu Val Asn Ile Ser Leu His Tyr Gln Lys Asp Glu
 110 115 120

gag ccc ctc ttc caa ctg aag aag gtc agg tct gtc aac tcc ttg atg 554
 Glu Pro Leu Phe Gln Leu Lys Lys Val Arg Ser Val Asn Ser Leu Met
 125 130 135

gtg gcc tct ctg act tac aaa gac aaa gtc tac ttg aat gtg acc act 602
 Val Ala Ser Leu Thr Tyr Lys Asp Lys Val Tyr Leu Asn Val Thr Thr
 140 145 150 155

gac aat acc tcc ctg gat gac ttc cat gtg aat ggc gga gaa ctg att 650
 Asp Asn Thr Ser Leu Asp Asp Phe His Val Asn Gly Gly Glu Leu Ile
 160 165 170

ctt atc cat caa aat cct ggt gaa ttc tgt gtc ctt tgagggctg 696
 Leu Ile His Gln Asn Pro Gly Glu Phe Cys Val Leu
 175 180

-continued

<210> SEQ ID NO 26
 <211> LENGTH: 183
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <400> SEQUENCE: 26

```

Met Glu Arg Val Gln Pro Leu Glu Glu Asn Val Gly Asn Ala Ala Arg
1           5           10          15

Pro Arg Phe Glu Arg Asn Lys Leu Leu Val Ala Ser Val Ile Gln
20          25           30

Gly Leu Gly Leu Leu Leu Cys Phe Thr Tyr Ile Cys Leu His Phe Ser
35          40           45

Ala Leu Gln Val Ser His Arg Tyr Pro Arg Ile Gln Ser Ile Lys Val
50          55           60

Gln Phe Thr Glu Tyr Lys Lys Glu Lys Gly Phe Ile Leu Thr Ser Gln
65          70           75           80

Lys Glu Asp Glu Ile Met Lys Val Gln Asn Asn Ser Val Ile Ile Asn
85          90           95

Cys Asp Gly Phe Tyr Leu Ile Ser Leu Lys Gly Tyr Phe Ser Gln Glu
100         105          110

Val Asn Ile Ser Leu His Tyr Gln Lys Asp Glu Glu Pro Leu Phe Gln
115         120          125

Leu Lys Lys Val Arg Ser Val Asn Ser Leu Met Val Ala Ser Leu Thr
130         135          140

Tyr Lys Asp Lys Val Tyr Leu Asn Val Thr Thr Asp Asn Thr Ser Leu
145         150          155          160

Asp Asp Phe His Val Asn Gly Gly Glu Leu Ile Leu Ile His Gln Asn
165         170          175

Pro Gly Glu Phe Cys Val Leu
180
  
```

<210> SEQ ID NO 27
 <211> LENGTH: 865
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (15)..(845)

<400> SEQUENCE: 27

```

cagcagagac gagg atg tgc gtt ggg gct cgg cgg ctg ggc cgc ggg ccg      50
      Met Cys Val Gly Ala Arg Arg Leu Gly Arg Gly Pro
      1           5           10

tgt gcg gct ctc ctc ctg ggc ctg ggg ctg agc acc gtg acg ggg      98
      Cys Ala Ala Leu Leu Leu Gly Leu Gly Leu Ser Thr Val Thr Gly
      15          20           25

ctc cac tgt gtc ggg gac acc tac ccc agc aac gac cgg tgc tgc cac      146
      Leu His Cys Val Gly Asp Thr Tyr Pro Ser Asn Asp Arg Cys Cys His
      30          35           40

gag tgc agg cca ggc aac ggg atg gtg agc cgc tgc agc cgc tcc cag      194
      Glu Cys Arg Pro Gly Asn Gly Met Val Ser Arg Cys Ser Arg Ser Gln
      45          50           55           60

aac acg gtg tgc cgt ccg tgc ggg ccg ggc ttc tac aac gac gtg gtc      242
      Asn Thr Val Cys Arg Pro Cys Gly Pro Gly Phe Tyr Asn Asp Val Val
      65          70           75

agc tcc aag ccg tgc aag ccc tgc acg tgg tgt aac ctc aga agt ggg      290
  
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-continued

Ser Ser Lys Pro Cys Lys Pro Cys Thr Trp Cys Asn Leu Arg Ser Gly	
80 85 90	
agt gag cgg aag cag ctg tgc acg gcc aca cag gac aca gtc tgc cgc	338
Ser Glu Arg Lys Gln Leu Cys Thr Ala Thr Gln Asp Thr Val Cys Arg	
95 100 105	
tgc cgg ggc acc cag ccc ctg gac agc tac aag cct gga gtt gac	386
Cys Arg Ala Gly Thr Gln Pro Leu Asp Ser Tyr Lys Pro Gly Val Asp	
110 115 120	
tgt gcc ccc tgc cct cca ggg cac ttc tcc cca ggc gac aac cag gcc	434
Cys Ala Pro Cys Pro Pro Gly His Phe Ser Pro Gly Asp Asn Gln Ala	
125 130 135 140	
tgc aag ccc tgg acc aac tgc acc ttg gct ggg aag cac acc ctg cag	482
Cys Lys Pro Trp Thr Asn Cys Thr Leu Ala Gly Lys His Thr Leu Gln	
145 150 155	
ccg gcc agc aat agc tcg gac gca atc tgt gag gac agg gac ccc cca	530
Pro Ala Ser Asn Ser Asp Ala Ile Cys Glu Asp Arg Asp Pro Pro	
160 165 170	
gcc acg cag ccc cag gag acc cag ggc ccc ccg gcc agg ccc atc act	578
Ala Thr Gln Pro Gln Glu Thr Gln Gly Pro Pro Ala Arg Pro Ile Thr	
175 180 185	
gtc cag ccc act gaa gcc tgg ccc aga acc tca cag gga ccc tcc acc	626
Val Gln Pro Thr Glu Ala Trp Pro Arg Thr Ser Gln Gly Pro Ser Thr	
190 195 200	
cgg ccc gtg gag gtc ccc ggg ggc cgt gcg gtt gcc gcc atc ctg ggc	674
Arg Pro Val Glu Val Pro Gly Gly Arg Ala Val Ala Ala Ile Leu Gly	
205 210 215 220	
ctg ggc ctg gtg ggg ctg ctg ggc ccc ctg gcc atc ctg ctg gcc	722
Leu Gly Leu Val Gly Leu Leu Gly Pro Leu Ala Ile Leu Leu Ala	
225 230 235	
ctg tac ctg ctc cgg agg gac cag agg ctg ccc ccc gat gcc cac aag	770
Leu Tyr Leu Leu Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys	
240 245 250	
ccc cct ggg gga ggc agt ttc cgg acc ccc atc caa gag gag cag gcc	818
Pro Pro Gly Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala	
255 260 265	
gac gcc cac tcc acc ctg gcc aag atc tgacctggc ccaccaaggt	865
Asp Ala His Ser Thr Leu Ala Lys Ile	
270 275	

<210> SEQ_ID NO 28

<211> LENGTH: 277

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 28

Met Cys Val Gly Ala Arg Arg Leu Gly Arg Gly Pro Cys Ala Ala Leu	
1 5 10 15	

Leu Leu Leu Gly Leu Gly Leu Ser Thr Val Thr Gly Leu His Cys Val	
20 25 30	

Gly Asp Thr Tyr Pro Ser Asn Asp Arg Cys Cys His Glu Cys Arg Pro	
35 40 45	

Gly Asn Gly Met Val Ser Arg Cys Ser Arg Ser Gln Asn Thr Val Cys	
50 55 60	

Arg Pro Cys Gly Pro Gly Phe Tyr Asn Asp Val Val Ser Ser Lys Pro	
65 70 75 80	

Cys Lys Pro Cys Thr Trp Cys Asn Leu Arg Ser Gly Ser Glu Arg Lys	
85 90 95	

-continued

Gln Leu Cys Thr Ala Thr Gln Asp Thr Val Cys Arg Cys Arg Ala Gly
 100 105 110
 Thr Gln Pro Leu Asp Ser Tyr Lys Pro Gly Val Asp Cys Ala Pro Cys
 115 120 125
 Pro Pro Gly His Phe Ser Pro Gly Asp Asn Gln Ala Cys Lys Pro Trp
 130 135 140
 Thr Asn Cys Thr Leu Ala Gly Lys His Thr Leu Gln Pro Ala Ser Asn
 145 150 155 160
 Ser Ser Asp Ala Ile Cys Glu Asp Arg Asp Pro Pro Ala Thr Gln Pro
 165 170 175
 Gln Glu Thr Gln Gly Pro Pro Ala Arg Pro Ile Thr Val Gln Pro Thr
 180 185 190
 Glu Ala Trp Pro Arg Thr Ser Gln Gly Pro Ser Thr Arg Pro Val Glu
 195 200 205
 Val Pro Gly Gly Arg Ala Val Ala Ala Ile Leu Gly Leu Gly Leu Val
 210 215 220
 Leu Gly Leu Leu Gly Pro Leu Ala Ile Leu Leu Ala Leu Tyr Leu Leu
 225 230 235 240
 Arg Arg Asp Gln Arg Leu Pro Pro Asp Ala His Lys Pro Pro Gly Gly
 245 250 255
 Gly Ser Phe Arg Thr Pro Ile Gln Glu Glu Gln Ala Asp Ala His Ser
 260 265 270
 Thr Leu Ala Lys Ile
 275

<210> SEQ_ID NO 29
 <211> LENGTH: 1599
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <221> NAME/KEY: CDS
 <222> LOCATION: (67)..(1596)

<400> SEQUENCE: 29

ccacaccaag cagcggctgg	ggggggggaaa gacgaggaaa	gaggaggaaa acaaaagctg	60
ctactt atg gaa gat aca aag gag	tct aac gtg aag aca	ttt tgc tcc	108
Met Glu Asp Thr Lys Glu Ser Asn Val	Lys Thr Phe Cys Ser		
1	5	10	
aag aat atc cta gcc atc ctt ggc	ttc tcc tct atc ata	gct gtg ata	156
Lys Asn Ile Leu Ala Ile Leu Gly	Phe Ser Ser Ile Ile Ala Val	Ile	
15	20	25	30
gct ttg ctt gct gtg ggg ttg acc	cag aac aaa gca ttg cca gaa aac		204
Ala Leu Ala Val Gly Leu Thr Gln Asn	Lys Ala Leu Pro Glu Asn		
35	40	45	
gtt aag tat ggg att gtg ctg	gat gcg ggt tct tct cac	aca agt tta	252
Val Lys Tyr Gly Ile Val Leu Asp	Ala Gly Ser Ser His	Thr Ser Leu	
50	55	60	
tac atc tat aag tgg cca gca gaa	aag gag aat gac aca ggc	gtg gtg	300
Tyr Ile Tyr Lys Trp Pro Ala Glu	Lys Glu Asn Asp Thr	Gly Val Val	
65	70	75	
cat caa gta gaa gaa tgc agg	gtt aaa ggt cct gga atc	tca aaa ttt	348
His Gln Val Glu Glu Cys Arg Val	Lys Gly Pro Gly Ile	Ser Lys Phe	
80	85	90	
gtt cag aaa gta aat gaa ata	ggc att tac ctg act gat	tgc atg gaa	396
Val Gln Lys Val Asn Glu Ile	Gly Ile Tyr Leu Thr Asp	Cys Met Glu	

-continued

95	100	105	110	
aga gct agg gaa gtg att cca agg tcc cag cac caa gag aca ccc gtt				444
Arg Ala Arg Glu Val Ile Pro Arg Ser Gln His Gln Glu Thr Pro Val				
115	120	125		
tac ctg gga gcc acg gca ggc atg cgg ttg ctc agg atg gaa agt gaa				492
Tyr Leu Gly Ala Thr Ala Gly Met Arg Leu Leu Arg Met Glu Ser Glu				
130	135	140		
gag ttg gca gac agg gtt ctg gat gtg gtc gag agg agc ctc agc aac				540
Glu Leu Ala Asp Arg Val Leu Asp Val Val Glu Arg Ser Leu Ser Asn				
145	150	155		
tac ccc ttt gac ttc cag ggt gcc agg atc att act ggc caa gag gaa				588
Tyr Pro Phe Asp Phe Gln Gly Ala Arg Ile Ile Thr Gly Gln Glu Glu				
160	165	170		
ggt gcc tat ggc tgg att act atc aac tat ctg ctg ggc aaa ttc agt				636
Gly Ala Tyr Gly Trp Ile Thr Ile Asn Tyr Leu Leu Gly Lys Phe Ser				
175	180	185	190	
cag aaa aca agg tgg ttc agc ata gtc cca tat gaa acc aat aat cag				684
Gln Lys Thr Arg Trp Phe Ser Ile Val Pro Tyr Glu Thr Asn Asn Gln				
195	200	205		
gaa acc ttt gga gct ttg gac ctt ggg gga gcc tct aca caa gtc act				732
Glu Thr Phe Gly Ala Leu Asp Leu Gly Gly Ala Ser Thr Gln Val Thr				
210	215	220		
ttt gta ccc caa aac cag act atc gag tcc cca gat aat gct ctg caa				780
Phe Val Pro Gln Asn Gln Thr Ile Glu Ser Pro Asp Asn Ala Leu Gln				
225	230	235		
ttt cgc ctc tat ggc aag gac tac aat gtc tac aca cat agc ttc ttg				828
Phe Arg Leu Tyr Gly Lys Asp Tyr Asn Val Tyr Thr His Ser Phe Leu				
240	245	250		
tgc tat ggg aag gat cag gca ctc tgg cag aaa ctg gcc aag gac att				876
Cys Tyr Gly Lys Asp Gln Ala Leu Trp Gln Lys Leu Ala Lys Asp Ile				
255	260	265	270	
cag gtt gca agt aat gaa att ctc agg gac cca tgc ttt cat cct gga				924
Gln Val Ala Ser Asn Glu Ile Leu Arg Asp Pro Cys Phe His Pro Gly				
275	280	285		
tat aag aag gta gtg aac gta agt gac ctt tac aag acc ccc tgc acc				972
Tyr Lys Val Val Asn Val Ser Asp Leu Tyr Lys Thr Pro Cys Thr				
290	295	300		
aag aga ttt gag atg act ctt cca ttc cag cag ttt gaa atc cag ggt				1020
Lys Arg Phe Glu Met Thr Leu Pro Phe Gln Gln Phe Glu Ile Gln Gly				
305	310	315		
att gga aac tat caa caa tgc cat caa agc atc ctg gag ctc ttc aac				1068
Ile Gly Asn Tyr Gln Gln Cys His Gln Ser Ile Leu Glu Leu Phe Asn				
320	325	330		
acc agt tac tgc cct tac tcc cag tgt gcc ttc aat ggg att ttc ttg				1116
Thr Ser Tyr Cys Pro Tyr Ser Gln Cys Ala Phe Asn Gly Ile Phe Leu				
335	340	345	350	
cca cca ctc cag ggg gat ttt ggg gca ttt tca gct ttt tac ttt gtg				1164
Pro Pro Leu Gln Gly Asp Phe Gly Ala Phe Ser Ala Phe Tyr Phe Val				
355	360	365		
atg aag ttt tta aac ttg aca tca gag aaa gtc tct cag gaa aag gtg				1212
Met Lys Phe Leu Asn Leu Thr Ser Glu Lys Val Ser Gln Glu Lys Val				
370	375	380		
act gag atg atg aaa aag ttc tgt gct cag cct tgg gag gag ata aaa				1260
Thr Glu Met Met Lys Lys Phe Cys Ala Gln Pro Trp Glu Glu Ile Lys				
385	390	395		
aca tct tac gct gga gta aag gag aag tac ctg agt gaa tac tgc ttt				1308
Thr Ser Tyr Ala Gly Val Lys Glu Lys Tyr Leu Ser Glu Tyr Cys Phe				

-continued

400	405	410	
tct ggt acc tac att ctc tcc ctc ctt ctg caa ggc tat cat ttc aca Ser Gly Thr Tyr Ile Leu Ser Leu Leu Gln Gly Tyr His Phe Thr 415 420 425 430			1356
gct gat tcc tgg gag cac atc cat ttc att ggc aag atc cag ggc agc Ala Asp Ser Trp Glu His Ile His Phe Ile Gly Lys Ile Gln Gly Ser 435 440 445			1404
gac gcc ggc tgg act ttg ggc tac atg ctg aac ctg acc aac atg atc Asp Ala Gly Trp Thr Leu Gly Tyr Met Leu Asn Leu Thr Asn Met Ile 450 455 460			1452
cca gct gag caa cca ttg tcc aca cct ctc tcc cac tcc acc tat gtc Pro Ala Glu Gln Pro Leu Ser Thr Pro Leu Ser His Ser Thr Tyr Val 465 470 475			1500
ttc ctc atg gtt cta ttc tcc ctg gtc ctt ttc aca gtg gcc atc ata Phe Leu Met Val Leu Phe Ser Leu Val Leu Phe Thr Val Ala Ile Ile 480 485 490			1548
ggc ttg ctt atc ttt cac aag cct tca tat ttc tgg aaa gat atg gta Gly Leu Leu Ile Phe His Lys Pro Ser Tyr Phe Trp Lys Asp Met Val 495 500 505 510			1596
tag			1599

<210> SEQ ID NO 30
 <211> LENGTH: 510
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 30

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

-continued

210	215	220
Pro Gln Asn Gln Thr Ile Glu Ser Pro Asp Asn Ala Leu Gln Phe Arg		
225	230	235
Leu Tyr Gly Lys Asp Tyr Asn Val Tyr Thr His Ser Phe Leu Cys Tyr		
245	250	255
Gly Lys Asp Gln Ala Leu Trp Gln Lys Leu Ala Lys Asp Ile Gln Val		
260	265	270
Ala Ser Asn Glu Ile Leu Arg Asp Pro Cys Phe His Pro Gly Tyr Lys		
275	280	285
Lys Val Val Asn Val Ser Asp Leu Tyr Lys Thr Pro Cys Thr Lys Arg		
290	295	300
Phe Glu Met Thr Leu Pro Phe Gln Gln Phe Glu Ile Gln Gly Ile Gly		
305	310	315
Asn Tyr Gln Gln Cys His Gln Ser Ile Leu Glu Leu Phe Asn Thr Ser		
325	330	335
Tyr Cys Pro Tyr Ser Gln Cys Ala Phe Asn Gly Ile Phe Leu Pro Pro		
340	345	350
Leu Gln Gly Asp Phe Gly Ala Phe Ser Ala Phe Tyr Phe Val Met Lys		
355	360	365
Phe Leu Asn Leu Thr Ser Glu Lys Val Ser Gln Glu Lys Val Thr Glu		
370	375	380
Met Met Lys Phe Cys Ala Gln Pro Trp Glu Glu Ile Lys Thr Ser		
385	390	395
Tyr Ala Gly Val Lys Glu Lys Tyr Leu Ser Glu Tyr Cys Phe Ser Gly		
405	410	415
Thr Tyr Ile Leu Ser Leu Leu Gln Gly Tyr His Phe Thr Ala Asp		
420	425	430
Ser Trp Glu His Ile His Phe Ile Gly Lys Ile Gln Gly Ser Asp Ala		
435	440	445
Gly Trp Thr Leu Gly Tyr Met Leu Asn Leu Thr Asn Met Ile Pro Ala		
450	455	460
Glu Gln Pro Leu Ser Thr Pro Leu Ser His Ser Thr Tyr Val Phe Leu		
465	470	475
Met Val Leu Phe Ser Leu Val Leu Phe Thr Val Ala Ile Ile Gly Leu		
485	490	495
Leu Ile Phe His Lys Pro Ser Tyr Phe Trp Lys Asp Met Val		
500	505	510

<210> SEQ ID NO 31
 <211> LENGTH: 8
 <212> TYPE: PRT
 <213> ORGANISM: Artificial sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Flag peptide
 <400> SEQUENCE: 31

Asp Tyr Lys Asp Asp Asp Asp Lys
 1
 5

<210> SEQ ID NO 32
 <211> LENGTH: 11
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: linker

-continued

<400> SEQUENCE: 32

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10

<210> SEQ ID NO 33
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: linker

<400> SEQUENCE: 33

Gly Ala Gly Gly Ala Gly Ser Gly Gly Gly Gly Ser
1 5 10

<210> SEQ ID NO 34
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: linker

<400> SEQUENCE: 34

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1 5 10

<210> SEQ ID NO 35
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: peptide

<400> SEQUENCE: 35

Gly Thr Pro Gly Thr Pro Gly Thr Pro
1 5

<210> SEQ ID NO 36
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: peptide

<400> SEQUENCE: 36

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Ser
20 25

What is claimed is:

1. A method of treating cardiovascular disease in a subject having cardiovascular disease, the method comprising administering to the subject a therapeutically effective amount of an antagonist, wherein the antagonist is selected from the group consisting of an IL-17 antagonist, IL-18 antagonist, 4-1BB antagonist, CD30 antagonist and an OX40 antagonist.

2. The method of claim 1, wherein the IL-17 antagonist is a soluble IL-17 receptor.

3. The method of claim 2, wherein the soluble IL-17 receptor is a fusion protein.

4. The method of claim 1, wherein the IL-17 antagonist is an antibody.

5. The method of claim 4, wherein the antibody specifically binds the IL-17 receptor.

6. The method of claim 4, wherein the antibody specifically binds IL-17.

7. The method of claim 4, wherein the antibody is a humanized antibody.

8. The method of claim 4, wherein the antibody is a single-chain antibody.

9. The method of claim 1, wherein the IL-17 antagonist is administered one or more times per week.

10. The method of claim 1, wherein the IL-17 antagonist is administered by subcutaneous injection.

11. The method of claim 1, wherein the IL-17 antagonist is administered in combination with one or more compounds selected from the group consisting of non-steroidal anti-

inflammatory drugs; analgesics; systemic steroids; antagonists of inflammatory cytokines; anti-inflammatory cytokines; chemotherapeutics; lipid-lowering drugs; blood pressure-regulating drugs; angiotensin-converting enzyme inhibitors and/or peroxisome proliferator-activated receptor ligands.

* * * * *