US 20070072249A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2007/0072249 A1**

Baum et al. (43) **Pub. Date: Mar. 29, 2007**

(54) **HUMAN CYTOKINE AND ALPHA-HELIX-CONTAINING POLYPEPTIDES**

(75) Inventors: **Peter R. Baum**, Seattle, WA (US); **Randal R. Ketchem**, Snohomish, WA (US); **Robert F. DuBose**, Bellevue, WA (US); **Steven R. Wiley**, Seattle, WA (US); **Keith N. Kerkof**, Seattle, WA (US)

Correspondence Address:
IMMUNEX CORPORATION
LAW DEPARTMENT
1201 AMGEN COURT WEST
SEATTLE, WA 98119 (US)

(73) Assignee: **Immunex Corporation**, Thousand Oaks, CA (US)

(21) Appl. No.: **11/634,751**

(22) Filed: **Dec. 6, 2006**

Related U.S. Application Data

(63) Continuation of application No. 10/165,772, filed on Jun. 7, 2002.

(60) Provisional application No. 60/296,951, filed on Jun. 8, 2001.

Publication Classification

(51) **Int. Cl.**

G01N 33/567	(2006.01)
C07H 21/04	(2006.01)
C12P 21/06	(2006.01)
C07K 14/705	(2006.01)
C07K 16/28	(2006.01)

(52) **U.S. Cl.** **435/7.2**; 435/69.1; 435/320.1; 435/366; 530/350; 530/388.22; 536/23.5

(57) **ABSTRACT**

This invention relates to new human alpha-helix-containing polypeptides, to methods of making such polypeptides, to methods of using them to treat immunological conditions, and to methods of identifying compounds that alter alpha-helix-containing polypeptide activities.

HUMAN CYTOKINE AND ALPHA-HELIX-CONTAINING POLYPEPTIDES

[0001] This application claims the benefit under 35 U.S.C. 119(e) of U.S. provisional application Ser. No. 60/296,951, filed 08 Jun. 2001, which is incorporated in its entirety by reference herein.

FIELD OF THE INVENTION

[0002] This invention relates to new alpha-helix-containing polypeptides similar to members of the human cytokine polypeptide family, and to methods of making and using them.

BACKGROUND OF THE INVENTION

[0003] The cytokine polypeptides are a related group of secreted polypeptides, having a three-dimensional structure characterized by a 'bundled' arrangement of four alpha helices. Members of this family of "four-alpha-helical-bundle" (4AHB) polypeptides also include hematopoietic growth factors, interferons, and hormones. The 4AHB cytokine polypeptides are all involved in regulating either the proliferation or the development of cells such as hematopoietic cells or immune cells from pluripotent stem cell precursors, with different combinations of cytokines affecting the formation of different cell types such as T cells, B cells, erythrocytes, megakaryocytes, mast cells, eosinophils, neutrophils, monocytes, macrophages, dendritic cells, and osteoclasts. However, some subgroups of these cytokines also affect biological activities of cells outside the hematopoietic or immune cell system, with their receptors widely expressed in different tissues (Nicola and Hilton, 1999, *Advances in Protein Chemistry* 52: 1-65).

[0004] Common structural features of the 4AHB cytokine family of polypeptides include signal sequences directing movement of the cytokine precursor polypeptide through the cell membrane to produce a secreted cytokine, or to the exterior surface of the cell membrane to produce a membrane-bound form of the cytokine that is then proteolytically cleaved and released from the cell. While most members of the 4AHB cytokine family are active as monomeric molecules, some form functional homodimers, or interact with soluble forms of cytokine receptors to form a heterodimeric molecule (Nicola and Hilton, 1999, *Advances in Protein Chemistry* 52: 1-65). The four alpha helices of the 4AHB cytokines, helices A through D, are arranged in an "up up down down" configuration (Kallen et al., 1999, *J Biol Chem* 274: 11859-11867). The A and D helices of the interleukin-6 (IL-6) cytokine have been found to include hydrophobic residues important in forming hydrophobic binding interactions with the IL-6 receptor alpha chain, interspersed with charged residues that are believed to form salt-bridge clusters with charged residues on the receptor chain, shielding the nearby hydrophobic residues from water molecules and stabilizing the cytokine-receptor interactions (Grotzinger et al., 1997, *PROTEINS: Structure, Function, and Genetics* 27: 96-109). The results of mutational studies identifying functional residues in the A and D helices of thrombopoietin (TPO), a hematopoietic cell growth factor of the 4AHB cytokine family (Jagerschmidt et al., 1998, *Biochem J* 333: 729-734), are consistent with this model of cytokine-receptor interaction.

[0005] Structurally, the 4AHB cytokine family can be divided into two groups: "short-chain" cytokines with shorter core alpha helices and two-strand beta-sheet structures in the inter-helical loops, and "long-chain" cytokines with longer core alpha helices and in many cases shorter alpha helices in the loop regions. The 4AHB cytokine family can also be subdivided on the basis of the type(s) of receptor complex(es) they interact with. For example, 4AHB cytokines may bind to a Type I or a Type H cytokine receptor which propagate regulatory signals through various members of the JAK and STAT families of intracellular signaling molecules, or they may bind to receptors with intrinsic tyrosine kinase activities (Nicola and Hilton, 1999, *Advances in Protein Chemistry* 52: 1-65); further, a variety of functional conformations are observed among the receptors for 4AHB cytokines, such as single-chain receptors, homodimers, heterodimers of an alpha 'cytokine-binding' chain and a beta 'signaling' chain that may also be present ('shared') in receptor complexes for other cytokines, and receptor complexes with three or more receptor chains (Cosman, 1993, *Cytokine* 5: 95-106).

[0006] Because of their roles in differentiation of hematopoietic and immune cells, 4AHB cytokine polypeptides are involved in a wide range of biological processes and associated disease states and conditions. For example, interaction of the 4AHB cytokine erythropoietin (EPO) with its receptor (a homodimer with an intracellular signaling domain that activates a pathway including JAK2 and STAT5) stimulates the proliferation and differentiation of erythrocyte precursor cells in adults, making EPO useful for treating anemia. The 4AHB cytokines thrombopoietin (TPO) and Granulocyte Colony-Stimulating Factor (G-CSF) also have hematopoiesis-stimulating activity. Other biological effects of 4AHB cytokines include regulation of neural cell and keratinocyte development, regulation of whole-body metabolism (an effect demonstrated by growth hormone (GH), prolactin (PRL), and leptin/OB, for example); stimulation of a proinflammatory response to infection or injury and of innate immunity (Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), IL-3, IL-5, IL-6, oncostatin M (OSM), and leukemia inhibitory factor (LIF), for example); anti-viral activity (interferons such as interferon alpha, beta, and gamma); and stimulation of acquired immunity and driving the differentiation of helper T cells toward Th1 cell fates (IL-12, for example) or Th2 cell fates (IL-2, IL-4, and IL-15, for example) (Nicola and Hilton, 1999, *Advances in Protein Chemistry* 52: 1-65).

[0007] In order to develop more effective treatments for conditions and diseases involving the proliferation or the development of cells from pluripotent stem cell precursors, information is needed about previously unidentified members of the 4AHB cytokine polypeptide family and other alpha-helix-containing immunomodulatory polypeptides, so that the characteristics and activities of structurally similar biologically active polypeptides can be ascertained and compared. In particular, there is a need for identification of previously unidentified human alpha-helix-containing polypeptides such as cytokine polypeptides.

SUMMARY OF THE INVENTION

[0008] The present invention is based upon the discovery of a set of alpha-helix-containing human polypeptides, including polypeptides similar to members of the human cytokine polypeptide family. Preferably, such polypeptides are isolated cytokine family polypeptides or isolated polypeptides having immunomodulatory activity.

[0009] The invention provides an isolated polypeptide consisting of, consisting essentially of, or more preferably, comprising an amino acid sequence selected from the group consisting of:

[0010] (a) an amino acid sequence selected from the group consisting of SEQ ID NOs 12 and 14;

[0011] (b) SEQ ID NO:16;

[0012] (c) an amino acid sequence selected from the group consisting of SEQ ID NO:16, SEQ ID NO:18, and SEQ ID NO:20;

[0013] (d) fragments of the amino acid sequences of any of (a)-(c) comprising at least 20 contiguous amino acids;

[0014] (e) fragments of the amino acid sequences of any of (a)-(c) comprising at least 30 contiguous amino acids;

[0015] (f) fragments of the amino acid sequences of any of (a)-(c) comprising alpha helix amino acid sequences;

[0016] (g) amino acid sequences comprising at least 20 amino acids and sharing amino acid identity with the amino acid sequences of any of (a)-(f), wherein the percent amino acid identity is selected from the group consisting of: at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97.5%, at least 99%, and at least 99.5%;

[0017] (h) an amino acid sequence of (g), wherein a polypeptide comprising said amino acid sequence of (g) binds to an antibody that also binds to a polypeptide comprising an amino acid sequence of any of (a)-(f); and

[0018] (i) the amino sequence of any of (a)-(h), wherein a polypeptide consisting of said amino acid sequence has immunomodulatory activity.

[0019] Other aspects of the invention are isolated nucleic acids encoding polypeptides of the invention, and isolated nucleic acids, preferably having a length of at least 15 nucleotides, that hybridize under conditions of moderate stringency to the nucleic acids encoding polypeptides of the invention. In preferred embodiments of the invention, such nucleic acids encode a polypeptide having immunomodulatory activity, or comprise a nucleotide sequence that shares nucleotide sequence identity with the nucleotide sequences of the nucleic acids of the invention, wherein the percent nucleotide sequence identity is selected from the group consisting of: at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97.5%, at least 99%, and at least 99.5%.

[0020] Further provided by the invention are expression vectors and recombinant host cells comprising at least one nucleic acid of the invention, and preferred recombinant host cells wherein said nucleic acid is integrated into the host cell genome.

[0021] Also provided is a process for producing a polypeptide encoded by the nucleic acids of the invention, comprising culturing a recombinant host cell under conditions promoting expression of said polypeptide, wherein the recombinant host cell comprises at least one-nucleic acid of the invention. A preferred process provided by the invention further comprises purifying said polypeptide. In another aspect of the invention, the polypeptide produced by said process is provided.

[0022] Further aspects of the invention are isolated antibodies that bind to the polypeptides of the invention, preferably monoclonal antibodies, also preferably humanized antibodies or humanized antibodies, and preferably wherein the antibody inhibits the activity of said polypeptides.

[0023] The invention additionally provides a method of designing an inhibitor of the polypeptides of the invention, the method comprising the steps of determining the three-dimensional structure of any such polypeptide, analyzing the three-dimensional structure for the likely binding sites of substrates, synthesizing a molecule that incorporates a predicted reactive site, and determining the polypeptide-inhibiting activity of the molecule.

[0024] In a further aspect of the invention, a method is provided for identifying compounds that alter immunomodulatory activity comprising

[0025] (a) mixing a test compound with a polypeptide of the invention; and

[0026] (b) determining whether the test compound alters the immunomodulatory activity of said polypeptide.

[0027] In another aspect of the invention, a method is provided identifying compounds that inhibit the binding activity of alpha-helix-containing polypeptides comprising

[0028] (a) mixing a test compound with a polypeptide of the invention and a binding partner of said polypeptide; and

[0029] (b) determining whether the test compound inhibits the binding activity of said polypeptide.

[0030] In preferred embodiments, the binding partner is a cell surface receptor that is a member of the immunoglobulin superfamily; more preferably, the binding partner is a member of the cytokine receptor family.

[0031] The invention also provides a method for increasing proliferation and/or differentiation of cells from pluripotent stem cell precursors, comprising providing at least one compound selected from the group consisting of the polypeptides of the invention and agonists of said polypeptides; with a preferred embodiment of the method further comprising increasing said activities in a patient by administering at least one polypeptide of the invention. Preferably, the pluripotent stem cell precursor is capable of differentiation into a cell type selected from the group consisting of T cells, B cells, erythrocytes, megakaryocytes, mast cells, eosinophils, neutrophils, monocytes, macrophages, dendritic cells, keratinocytes, and osteoclasts.

[0032] Further provided by the invention is a method for decreasing proliferation and/or differentiation of cells from pluripotent stem cell precursors, comprising providing at least one antagonist of the polypeptides of the invention; with a preferred embodiment of the method further comprising decreasing said activities in a patient by administering at least one antagonist of the polypeptides of the invention, and with a further preferred embodiment wherein the antagonist is an antibody that inhibits the activity of any of said polypeptides.

[0033] The invention additionally provides a method for increasing the number of receptor-bearing cells or their developmentally committed progeny, through increased cell

proliferation and/ or altered cell differentiation, comprising contacting said receptor-bearing cells with polypeptides of the invention or agonists thereof. In preferred embodiments, the receptor-bearing cells are pluripotent cells, and in further preferred embodiments, the receptor-bearing cells are cells of the hematopoietic system.

[0034] In other aspects of the invention, methods are provided for treating cytopenias for receptor-bearing cells or their developmentally committed progeny, comprising administering to a patient a therapeutically effective amount of one or more polypeptides of the invention or agonists thereof. In preferred embodiments, the patient is a human patient; and in further preferred embodiments, the cytopenia is a disease affecting hematopoietic cells. Methods are also provided for treating the hypoproliferation of receptor-bearing cells or their developmentally committed progeny, comprising administering to a patient a therapeutically effective amount of one or more antagonists of polypeptides of the invention. In preferred embodiments, the patient is a human patient; and in further preferred embodiments, the hypoproliferation is a cancerous or metastatic condition; and more preferably the hypoproliferation is a lymphoproliferation such as leukemia.

[0035] Also encompassed within the scope of the invention are methods for increasing immune activity against pathogens and/or tumors by increasing specific subclasses of immune cells with increased effector functions, comprising administering to a patient a therapeutically effective amount of one or more polypeptides of the invention or agonists thereof. In preferred embodiments, the patient is a human patient; and in a further preferred embodiment, the increased effector function is increased cytolytic lymphocyte function against virally infected or cancerous cells.

[0036] A further embodiment of the invention provides a use for the polypeptides of the invention in the preparation of a medicament for treating cytopenias for receptor-bearing cells or their developmentally committed progeny; with a preferred embodiment wherein the cytopenia is anemia.

[0037] The invention further provides methods for producing information comprising the identity of one or more compounds that alter the biological activity of alpha-helix-containing polypeptides, the method comprising using assay methods of the invention to identify one or more compounds that alter immunomodulatory activity. In one preferred embodiment, the compound decreases the biological activity of one or more alpha-helix-containing polypeptides, and in another distinct embodiment, the compound increases the biological activity of one or more alpha-helix-containing polypeptides. Preferably the biological activity of alpha-helix-containing polypeptides is selected from the group consisting of stimulation of the proliferation and/or differentiation of cells from pluripotent stem cell precursors, and the binding of alpha-helix-containing polypeptides to binding partners such as cytokine receptors. Also provided by the invention is the information produced according to these methods, said information comprising the identity of a compound that alters the biological activity of alpha-helix-containing polypeptides, and preferably embodied in a storage medium selected from the group consisting of the brains of living organisms, paper, magnetic tape, optical tape, floppy disks, compact disks, computer system hard drives, and computer memory units. In a further aspect, the invention provides a database comprising said information, wherein the information is preferably embodied in a computer-readable medium, and a separate embodiment wherein the information is embodied in a human-readable medium.

[0038] Additionally provided by the invention is a computer system comprising a database containing records pertaining to a plurality of compounds, wherein the records comprise results of an assay of the invention, and a user interface allowing a user to access information regarding the plurality of compounds. In another aspect of the invention, a computer system is provided for storing and retrieving data on a plurality of compounds, the computer system comprising:

[0039] (a) input means for entering data for the compounds into a storage medium;

[0040] (b) a processor for creating an individual record for each compound, the processor assigning specific identifying values for each compound;

[0041] (c) means for selecting one or more of the records based on results in an assay; and

[0042] (d) means for transmitting information in the record or records to an output device to produce a report; preferably a report in human-readable form, and wherein the computer system preferably further comprises a video display unit. The invention also provides a method of using the computer system of the invention to select one or more compounds for testing from a plurality of compounds having records stored in a database, the method comprising: displaying a list of said records or a field for entering information identifying one or more of said records; and selecting one or more of the records from the list or the record or records identified by entering information in the field. Further, the invention provides a method of operating a computer system for analyzing compounds that modulate the interaction of alpha-helix-containing polypeptides and their binding partners, the method comprising:

[0043] (a) entering data relating to a plurality of compounds into a storage medium;

[0044] (b) processing the data to create an individual record for each compound;

[0045] (c) testing compounds for the ability to modulate binding of one or more alpha-helix-containing polypeptides to one or more binding partners; and

[0046] (d) communicating results from the testing into the storage medium such that results for each compound are associated with the individual record for that compound; wherein in one embodiment the storage medium comprises one or more computer memory units, and in another embodiment the computer system further comprises a video display unit. In yet another aspect of the invention, a database is provided comprising records generated according to the methods of the invention, and a method is provided for selecting compounds that modulate the interaction of alpha-helix-containing polypeptides and their binding partners, comprising compiling said database, analyzing the testing results, and selecting one or more compounds.

DETAILED DESCRIPTION OF THE INVENTION

Similarities in Structure to Cytokine Family Members

[0047] The typical structural elements common to members of the 4AHB cytokine polypeptide family include a signal sequence and four 'core' alpha helices. These helices and the inter-helix regions are referred to, in N-to-C order, as helix A, loop AB, helix B, loop BC, helix C, loop CD, and helix D. The locations of these helices within alpha-helix-containing polypeptides can be determined by using the GeneFold program (Tripos, Inc., St. Louis, Mo.; Jaroszewski et al., 1998, *Prot Sci* 7: 1431-1440), a protein threading program that overlays a query protein sequence onto structural representatives of the Protein Data Bank (PDB). As described above, four alpha helix bundle (4AHB) cytokine family members are characterized by a particular three-dimensional structure; this four-helical structure can be predicted from their primary amino acid sequences by using protein-threading algorithms such as GeneFold. To use GeneFold to chracterize new members of a protein family, the new protein sequence is entered into the program, which assigns a probability score that reflects how well it folds onto known protein structures ("template" structures) that are present in the GeneFold database. For scoring, GeneFold relies on primary amino acid sequence similarity, burial patterns of residues, local interactions, and secondary structure comparisons. The GeneFold program folds (or threads) the amino acid sequence onto all of the template structures in a database of protein folds, which includes the solved structures for several human cytokine/growth factor polypeptides such as Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Granulocyte Colony-Stimulating Factor (G-CSF), and interferon-alpha 2 (IFN-alpha2). For each comparison, three different scores are calculated—the 'sq', 'br', and 'tt' scores respectively—based on (i) sequence only; (ii) sequence plus local conformation preferences plus burial terms; and (iii) sequence plus local conformation preferences plus burial terms plus secondary structure. In each instance, the program determines the optimal alignment, calculates the probability (P-value) that this degree of alignment occurred by chance, and reports the inverse of the P-value as the score. These scores therefore reflect the degree to which the new protein matches the various reference structures and are useful for characterizing a newly identified alpha-helix-containing polypeptide by its structural relationships to known cytokines. Note that there may be an overlap between the predicted extent of helix B and that of helix C, resulting from the loop BC region between these helices assuming an extended conformation in some GeneFold template structures and a helical structure in others, consistent with the loop BC region being a flexible region that can have varied conformations in different 4AHB cytokines. The skilled artisan will recognize that the boundaries of the regions of alpha-helix-containing polypeptides described above are approximate and that the precise boundaries of such domains, as for example the boundaries of the signal sequence or the transmembrane domain (which can be predicted by using computer programs available for that purpose), can also differ from member to member within a polypeptide family.

[0048] The cytokine polypeptide family is well conserved with respect to its three-dimensional structure as described above, and there is considerable inter-species conservation for particular family members: human growth hormone (GH) is very similar to other mammalian GH polypeptides, for example. However, different members of the family generally have a lesser degree of primary amino acid sequence conservation (human G-CSF as compared to human IFN-alpha2, for instance), with an exception being closely-related polypeptides that probably arose from recent gene duplications, such as that described for SEQ ID NO:8 (or NO:9) and NO:10 as described further below.

[0049] When analyzed using GeneFold as described above, the polypeptides of SEQ ID NOs 12 and 14 are predicted to contain at least three alpha helical regions; these alpha helical regions are located approximately at amino acids 39 through 63 of SEQ ID NO:12 or SEQ ID NO:14; approximately at amino acids 73 through 117 of SEQ ID NO:12 or SEQ ID NO:14; and approximately at amino acids 126 through 164 of SEQ ID NO:12 or SEQ ID NO:14. This third alpha helical region overlaps with the first of three transmembrane regions that are predicted for the polypeptides of SEQ ID NOs 12 and 14 at the following approximate positions in both polypeptides: amino acids 143 through 165, amino acids 196 through 214, and amino acids 226 through 245.

[0050] The polypeptides of SEQ ID NOs 16 and 18 are related human polypeptides encoded by sequences located at human chromosome 22q11.1. (on overlapping genomic contigs with GenBank accession numbers AC000097.1 and AC006549.28, for example). Although the publicly available human genomic data indicates only one genomic region encoding the polypeptide of SEQ ID NO:16, analysis of genomic data available from Celera Genomics indicates a second locus, highly similar in sequence, encoding the polypeptide of SEQ ID NO:18. These polypeptides are encoded by several exons and show additional variation relative to each other due to alternative splicing. The polypeptide of SEQ ID NO:16 has two long alpha helices at amino acids 38 through 97 and amino acids 133 through 227 of SEQ ID NO:16. The C-terminal end of the second alpha helix overlaps with the transmembrane domain predicted for this polypeptide at amino acids 213 through 234 of SEQ ID NO:16. The polypeptide of SEQ ID NO:18 has a similar structure to that of SEQ ID NO:16.

Biological Activities and Functions of Cytokine and Alpha-Helix-Containing Polypeptides

[0051] Typical biological activities or functions associated with alpha-helix-containing polypeptides such as cytokine polypeptides are stimulation of the proliferation and/or differentiation of cells from pluripotent stem cell precursors. Alpha-helix-containing polypeptides having stimulation of cell proliferation activity bind receptor polypeptides. The receptor-binding activity is associated with domains comprising alpha helices of alpha-helix-containing polypeptides. Thus, for uses requiring receptor-binding activity, preferred alpha-helix-containing polypeptides include those having one or more alpha helices and exhibiting stimulation of cell proliferation activity. Preferred alpha-helix-containing polypeptides further include oligomers or fusion polypeptides comprising at least one alpha helix portion of one or more alpha-helix-containing polypeptides, and fragments of any of these polypeptides that have stimulation of cell proliferation activity. The receptor-dependent stimulation of cell proliferation activity of alpha-helix-containing polypep-

tides can be determined, for example, in a cell proliferation assay using BAF cells transfected with nucleic acid constructs directing the expression of receptor polypeptide chains (see, for example, FIG. 6of Kallen et al., 1999, *J Biol Chem* 274: 11859-11867). Alternatively, the effect that treatment of cells with alpha-helix-containing polypeptides has on activation of intracellular signaling pathways can be assayed by measuring the phosphorylation of receptor polypeptide chains or other targets of signaling pathway kinases such as targets of JAK family members (see, for example, FIG. 2of Kallen et al., 1999, *J Biol Chem* 274: 11859-11867). Alpha-helix-containing polypeptides having stimulation of cell proliferation activity preferably have at least 10% (more preferably, at least 25%, and most preferably, at least 50%) of the maximal stimulation of cell proliferation activity of IL-6 as measured in FIG. **6A**of Kallen et al., 1999, *J Biol Chem* 274: 11859-11867. Alpha-helix-containing polypeptides having stimulation of intracellular signalling activity preferably have at least 10% (more preferably, at least 25%, and most preferably, at least 50%) of the maximal phosphorylation of intracellular signaling pathway components activity of IL-6 as measured in FIG. **2A**of Kallen et al., 1999, *J Biol Chem* 274: 11859-11867. The term "immunomodulatory activity," as used herein, includes any one or more of the following: stimulation of cell proliferation activity and phosphorylation of intracellular signaling pathway components activity, as well as the ex vivo and in vivo activities of alpha-helix-containing polypeptides. The degree to which individual alpha-helix-containing polypeptides and fragments and other derivatives of these polypeptides exhibit these activities can be determined by standard assay methods, particularly assays such as those disclosed in Kallen et al., 1999, J Biol Chem 274: 11859-11867. Additional exemplary assays are disclosed herein; those of skill in the art will appreciate that other, similar types of assays can be used to measure alpha-helix-containing polypeptide biological activities.

[**0052**] Another aspect of the biological activity of alpha-helix-containing polypeptides is the ability to bind particular binding partners such as, for example, cell surface receptors that are members of the immunoglobulin superfamily, and more particularly members of the cytokine receptor family. The term "binding partner," as used herein, includes ligands, receptors, substrates, antibodies, other alpha-helix-containing polypeptides, the same alpha-helix-containing polypeptide (in the case of homotypic interactions or formation of multimers), and any other molecule that interacts with an alpha-helix-containing polypeptide through contact or proximity between particular portions of the binding partner and the alpha-helix-containing polypeptide. Because the alpha helices of alpha-helix-containing polypeptides are likely to be involved in the interaction with the receptor or binding partner, mutations of hydrophobic or charged residues within these helices are expected to alter the binding of alpha-helix-containing polypeptides to receptor polypeptides; such mutations are likely to disrupt binding of alpha-helix-containing polypeptides to receptor but may increase the strength of this interaction. By binding to one or more components of a receptor complex, or by binding to some components but not others, an altered alpha-helix-containing polypeptide would likely prevent binding by the native alpha-helix-containing polypeptide(s), and so act in a dominant negative fashion to inhibit the biological activities mediated via binding of alpha-helix-containing polypeptides to receptors (see, for example, Tables I and II of interactions in Grötzinger et al., 1997, *PROTEINS: Structure, Function, and Genetics* 27: 96-109). Suitable assays to detect or measure the binding between alpha-helix-containing polypeptides and their binding partners are well known to those of skill in the art and are described herein.

[**0053**] Alpha-helix-containing polypeptides are involved in diseases or conditions that share as a common feature proliferation and/or differentiation of cells from pluripotent stem cell precursors, or defects in such proliferative and/or developmental processes, in their etiology. Blocking or inhibiting the interactions between alpha-helix-containing polypeptides and their substrates, ligands, receptors, binding partners, and or other interacting polypeptides is an aspect of the invention and provides methods for treating or ameliorating diseases and conditions involving excess proliferation and/or differentiation of cells from pluripotent stem cell precursors, through the use of inhibitors of immunomodulatory activity. Examples of such inhibitors or antagonists are described in more detail below. For conditions involving inadequate proliferation and/or differentiation of cells from pluripotent stem cell precursors, methods of treating or ameliorating these conditions comprise increasing the amount or activity of alpha-helix-containing polypeptides by providing isolated alpha-helix-containing polypeptides or active fragments or fusion polypeptides thereof, or by providing compounds (agonists) that activate endogenous or exogenous alpha-helix-containing polypeptides. Additional uses for alpha-helix-containing polypeptides include diagnostic reagents for conditions and diseases involving the proliferation or the development of cells from pluripotent stem cell precursors, research reagents for investigation of proliferation and/or differentiation of cells from pluripotent stem cell precursors, or as a carrier/targeting polypeptide to deliver therapeutic agents to cells expressing receptors or binding partners for such alpha-helix-containing polypeptides.

Alpha-Helix-Containing Polypeptides

[**0054**] An alpha-helix-containing polypeptide is a polypeptide that shares a sufficient degree of amino acid identity or similarity, and/or similarity in secondary structure, to other alpha-helix-containing polypeptides such as members of the cytokine family of polypeptides, to (A) be identified by those of skill in the art as a polypeptide likely to share particular structural domains with alpha-helix-containing polypeptides and/or (B) have biological activities in common with alpha-helix-containing polypeptides and/or (C) bind to antibodies that also specifically bind to other alpha-helix-containing polypeptides. Alpha-helix-containing polypeptides may be isolated from naturally occurring sources, or have the same structure as naturally occurring alpha-helix-containing polypeptides, or may be produced to have structures that differ from naturally occurring alpha-helix-containing polypeptides. Polypeptides derived from any alpha-helix-containing polypeptide by any type of alteration (for example, but not limited to, insertions, deletions, or substitutions of amino acids; changes in the state of glycosylation of the polypeptide; refolding or isomerization to change its three-dimensional structure or self-association state; and changes to its association with other polypeptides or molecules) are also alpha-helix-containing family polypeptides. Therefore, the polypeptides provided by the invention include polypeptides characterized by amino acid

sequences similar to those of the alpha-helix-containing polypeptides described herein, but into which modifications are naturally provided or deliberately engineered. A polypeptide that shares biological activities in common with alpha-helix-containing polypeptides of the invention is a polypeptide having immunomodulatory activity. Examples of biological activities exhibited by alpha-helix-containing polypeptides of the invention include, without limitation, stimulation of proliferation and/or differentiation of cells from pluripotent stem cell precursors.

[0055] The present invention provides both full-length and mature forms of alpha-helix-containing polypeptides. Full-length polypeptides are those having the complete primary amino acid sequence of the polypeptide as initially translated. The amino acid sequences of full-length polypeptides can be obtained, for example, by translation of the complete open reading frame ("ORF") of a cDNA molecule. Several full-length polypeptides may be encoded by a single genetic locus if multiple mRNA forms are produced from that locus by alternative splicing or by the use of multiple translation initiation sites. The "mature form" of a polypeptide refers to a polypeptide that has undergone post-translational processing steps such as cleavage of the signal sequence or proteolytic cleavage to remove a prodomain. Multiple mature forms of a particular full-length polypeptide may be produced, for example by cleavage of the signal sequence at multiple sites, or by differential regulation of proteases that cleave the polypeptide. The mature form(s) of such polypeptide may be obtained by expression, in a suitable mammalian cell or other host cell, of a nucleic acid molecule that encodes the full-length polypeptide. The sequence of the mature form of the polypeptide may also be determinable from the amino acid sequence of the full-length form, through identification of signal sequences or protease cleavage sites. The alpha-helix-containing polypeptides of the invention also include those that result from post-transcriptional or post-translational processing events such as alternate mRNA processing which can yield a truncated but biologically active polypeptide, for example, a naturally occurring soluble form of the polypeptide. Also encompassed within the invention are variations attributable to proteolysis such as differences in the N- or C-termini upon expression in different types of host cells, due to proteolytic removal of one or more terminal amino acids from the polypeptide (generally from 1-5 terminal amino acids).

[0056] The invention further includes alpha-helix-containing polypeptides with or without associated native-pattern glycosylation. Polypeptides expressed in yeast or mammalian expression systems (e.g., COS-1 or CHO cells) can be similar to or significantly different from a native polypeptide in molecular weight and glycosylation pattern, depending upon the choice of expression system. Expression of polypeptides of the invention in bacterial expression systems, such as *E. coli,* provides non-glycosylated molecules. Further, a given preparation can include multiple differentially glycosylated species of the polypeptide. Glycosyl groups can be removed through conventional methods, in particular those utilizing glycopeptidase. In general, glycosylated polypeptides of the invention can be incubated with a molar excess of glycopeptidase (Boehringer Mannheim).

[0057] Species homologues of alpha-helix-containing polypeptides and of nucleic acids encoding them are also provided by the present invention. As used herein, a "species homologue" is a polypeptide or nucleic acid with a different species of origin from that of a given polypeptide or nucleic acid, but with significant sequence similarity to the given polypeptide or nucleic acid, as determined by those of skill in the art. Species homologues may be isolated and identified by making suitable probes or primers from polynucleotides encoding the amino acid sequences provided herein and screening a suitable nucleic acid source from the desired species. The invention also encompasses allelic variants of alpha-helix-containing polypeptides and nucleic acids encoding them; that is, naturally-occurring alternative forms of such polypeptides and nucleic acids in which differences in amino acid or nucleotide sequence are attributable to genetic polymorphism (allelic variation among individuals within a population).

[0058] Fragments of the alpha-helix-containing polypeptides of the present invention are encompassed by the present invention and may be in linear form or cyclized using known methods, for example, as described in H. U. Saragovi, et al., Bio/Technology 10, 773-778 (1992) and in R. S. McDowell, et al., J. Amer. Chem. Soc. 114 9245-9253 (1992). Polypeptides and polypeptide fragments of the present invention, and nucleic acids encoding them, include polypeptides and nucleic acids with amino acid or nucleotide sequence lengths that are 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 a alpha-helix-containing polypeptide and have 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 that alpha-helix-containing polypeptide or encoding nucleic acid, where sequence identity is determined by comparing the amino acid sequences of the polypeptides when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are polypeptides and polypeptide fragments, and nucleic acids encoding them, that contain or encode a segment preferably comprising at least 8, or at least 10, or preferably at least 15, or more preferably at least 20, or still more preferably at least 30, or most preferably at least 40 contiguous amino acids. Such polypeptides and polypeptide fragments may also contain a segment that shares at least 70% 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 any such segment of any of the alpha-helix-containing polypeptides, where sequence identity is determined by comparing the amino acid sequences of the polypeptides when aligned so as to maximize overlap and identity while minimizing sequence gaps. The percent identity can be determined by visual inspection and mathematical calculation. Alternatively, the percent identity of two amino acid or two nucleic acid sequences can be determined by comparing sequence information using the GAP computer program, version 6.0 described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available from the University of Wisconsin Genetics Computer Group (UWGCG). The preferred default parameters for the GAP program include: (1) a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted 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; (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps. Other programs used by those skilled in the art of sequence comparison may also be used, such as, for example, the BLASTN program version 2.0.9, available for use via the National Library of Medicine website: ncbi.nlm.riih.gov/gorf/wblast2.cgi, or the UW-BLAST 2.0 algorithm. Standard default parameter settings for UW-BLAST 2.0 are described at the following Internet webpage: blast.wustl.edu/blast/README.html#References. In addition, the BLAST algorithm uses the BLOSUM62 amino acid scoring matix, and optional parameters that may 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 & Federhen (Computers and Chemistry, 1993); also see Wootton J C and Federhen S, 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 & 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.

[0059] The present invention also provides for soluble forms of alpha-helix-containing polypeptides comprising certain fragments or domains of these polypeptides, and particularly those comprising the extracellular domain or one or more fragments of the extracellular domain. Soluble polypeptides are polypeptides that are capable of being secreted from the cells in which they are expressed. In such forms part or all of the intracellular and transmembrane domains of the polypeptide are deleted such that the polypeptide is fully secreted from the cell in which it is expressed. The intracellular and transmembrane domains of polypeptides of the invention can be identified in accordance with known techniques for determination of such domains from sequence information. Soluble alpha-helix-containing polypeptides also include those polypeptides which include part of the transmembrane region, provided that the soluble alpha-helix-containing polypeptide is capable of being secreted from a cell, and preferably retains immunomodulatory activity. Soluble alpha-helix-containing polypeptides further include oligomers or fusion polypeptides comprising the extracellular portion of at least one alpha-helix-containing polypeptide, and fragments of any of these polypeptides that have immunomodulatory activity. 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 alpha-helix-containing polypeptides 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.

[0060] In another aspect of the invention, preferred polypeptides comprise various combinations of alpha-helix-containing polypeptide domains, such as Helix A and Helix D. Accordingly, polypeptides of the present invention and nucleic acids encoding them include those comprising or encoding two or more copies of a domain such as Helix A, two or more copies of a domain such as Helix D, or at least one copy of each domain, and these domains may be presented in any order within such polypeptides.

[0061] Further modifications in the peptide or DNA sequences can be made by those skilled in the art using known techniques. Modifications of interest in the polypeptide sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid. For example, one or more of the cysteine residues may 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 polypeptide 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 by replaced with another amino acid, such as Ala. Known procedures for inactivating N-glycosylation sites in polypeptides include those described in U.S. Pat. No. 5,071,972 and EP 276,846. Additional variants within the scope of the invention include 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. Conjugates comprising diagnostic (detectable) or therapeutic agents attached thereto are contemplated herein. Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the polypeptide or a substantial equivalent thereof. One example is a variant that binds with essentially 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.

[0062] Other 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. 5,011,912 and in Hopp et al., *Bio/Technology* 6:1204, 1988. One such peptide is the FLAG® peptide, 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. Further, fusion polypeptides can comprise peptides added to facilitate purification and identification (referred to as tag peptides). Additional, useful tag peptides include, for example, green fluorescent protein (GFP; Chalfie et al., Science 263:802, 1994), an N-terminal peptide that contains recognition sites for a monoclonal antibody, a specific endopeptidase, and a site-specific protein kinase (PKA; Blanar and Rutter, Science 256:1014, 1992), birA (Altman et al., Science 274:94, 1996).and glutathione S transferase (GST: Smith and Johnson, Gene 67:31, 1988).

[0063] Encompassed by the invention are oligomers or fusion polypeptides that contain a alpha-helix-containing polypeptide, one or more fragments of alpha-helix-containing polypeptides, or any of the derivative or variant forms of alpha-helix-containing polypeptides as disclosed herein. In particular embodiments, the oligomers comprise soluble alpha-helix-containing polypeptides. Oligomets can be in the form of covalently linked or non-covalently-linked multimers, including dimers, trimers, or higher oligomers. In one aspect of the invention, the oligomers maintain the binding ability of the polypeptide components and provide therefor, bivalent, trivalent, etc., binding sites. In an alternative embodiment the invention is directed to oligomers comprising multiple alpha-helix-containing 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.

[0064] In embodiments where variants of the alpha-helix-containing polypeptides are constructed to include a membrane-spanning domain, they will form a Type I membrane polypeptide. Membrane-spanning alpha-helix-containing polypeptides can be fused with extracellular domains of receptor polypeptides for which the ligand is known. Such fusion polypeptides can then be manipulated to control the intracellular signaling pathways triggered by the membrane-spanning alpha-helix-containing polypeptide. Alpha-helix-containing polypeptides that span the cell membrane can also be fused with agonists or antagonists of cell-surface receptors, or cellular adhesion molecules to further modulate alpha-helix-containing polypeptide intracellular effects. In another aspect of the present invention, interleukins can be situated between the preferred alpha-helix-containing polypeptide fragment and other fusion polypeptide domains.

[0065] Immunoulobulin-based Oligomers. The polypeptides of the invention or fragments thereof may be fused to molecules such as immunoglobulins for many purposes, including increasing the valency of polypeptide binding sites. For example, fragments of a alpha-helix-containing polypeptide may be fused directly or through linker sequences to the Fc portion of an immunoglobulin. For a bivalent form of the polypeptide, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a polypeptide-IgM fusion would generate a decavalent form of the polypeptide of the invention. The term "Fc polypeptide" as used herein includes native and mutein 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) has 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). Methods for preparation and use of immunoglobulin-based oligomers are well known in the art. One embodiment of the present invention is directed to a dimer comprising two fusion polypeptides created by fusing a polypeptide of the invention to an Fc polypeptide derived from an antibody. A gene fusion encoding the polypeptide/Fc fusion polypeptide is inserted into an appropriate expression vector. Polypeptide/Fc fusion polypeptides are expressed in host cells transformed with the recombinant expression vector, and allowed to assemble much like antibody molecules, whereupon interchain 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. Another useful Fc polypeptide is the Fc mutein 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 mutein 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 mutein 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 alpha-helix-containing extracellular regions.

[0066] Peptide-linker Based Oligomers. Alternatively, the oligomer is a fusion polypeptide comprising multiple alpha-helix-containing 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 alpha-helix-containing 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.

[0067] Leucine-Zippers. Another method for preparing the oligomers of the invention involves use of a leucine zipper. 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 (Landschulz et al., *Science* 240:1759, 1988), 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.

[0068] Other fragments and derivatives of the sequences of polypeptides which would be expected to retain polypeptide activity in whole or in part and may thus be useful for screening or other immunological methodologies may also be made by those skilled in the art given the disclosures herein. Such modifications are believed to be encompassed by the present invention.

Nucleic Acids Encoding Alpha-Helix-Containing Polypeptides

[0069] Encompassed within the invention are nucleic acids encoding alpha-helix-containing polypeptides. These nucleic acids can be identified in several ways, including isolation of genomic or cDNA molecules from a suitable source. Nucleotide sequences corresponding to the amino acid sequences described herein, to be used as probes or primers for the isolation of nucleic acids or as query sequences for database searches, can be obtained by "back-translation" from the amino acid sequences, or by identification of regions of amino acid identity with polypeptides for which the coding DNA sequence has been identified. The well-known polymerase chain reaction (PCR) procedure can be employed to isolate and amplify a DNA sequence encoding a alpha-helix-containing polypeptide or a desired combination of alpha-helix-containing polypeptide fragments. Oligonucleotides that define the desired termini of the combination of DNA fragments are employed as 5' and 3' primers. The oligonucleotides can additionally contain recognition sites for restriction endonucleases, to facilitate insertion of the amplified combination of DNA fragments into an expression vector. PCR techniques are described in Saiki et al., *Science* 239:487 (1988); *Recombinant DNA Methodology,* Wu et al., eds., Academic Press, Inc., San Diego (1989), pp. 189-196; and *PCR Protocols: A Guide to Methods and Applications,* Innis et. al., eds., Academic Press, Inc. (1990).

[0070] Nucleic acid molecules of the invention include DNA and RNA in both single-stranded and double-stranded form, as well as the corresponding complementary sequences. DNA includes, for example, cDNA, genomic DNA, chemically synthesized DNA, DNA amplified by PCR, and combinations thereof. The nucleic acid molecules of the invention include full-length genes or cDNA molecules as well as a combination of fragments thereof. The nucleic acids of the invention are preferentially derived from human sources, but the invention includes those derived from non-human species, as well.

[0071] An "isolated nucleic acid" is a nucleic acid that has been separated from adjacent genetic sequences present in the genome of the organism from which the nucleic acid was isolated, in the case of nucleic acids isolated from naturally-occurring sources. In the case of nucleic acids synthesized enzymatically from a template or chemically, such as PCR products, cDNA molecules, or oligonucleotides for example, it is understood that the nucleic acids resulting from such processes are isolated nucleic acids. An isolated nucleic acid molecule refers to a nucleic acid molecule in the form of a separate fragment or as a component of a larger nucleic acid construct. In one preferred embodiment, the invention relates to certain isolated nucleic acids that are substantially free from contaminating enddgenous material. The nucleic acid molecule has preferably been derived from DNA or RNA isolated at least once in substantially pure form and in a quantity or concentration enabling identification, manipulation, and recovery of its component nucleotide sequences by standard biochemical methods (such as those outlined in Sambrook et al., *Molecular Cloning: A Laboratory Manual,* 2nd sed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1989)). Such sequences are preferably provided and/or constructed in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, that are typically present in eukaryotic genes. Sequences of non-translated DNA can be present 5' or 3' from an open reading frame, where the same do not interfere with manipulation or expression of the coding region.

[0072] The present invention also includes nucleic acids that hybridize under moderately stringent conditions, and more preferably highly stringent conditions, to nucleic acids encoding alpha-helix-containing polypeptides described herein. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by Sambrook, J., E. F. Fritsch, and T. 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, F. M. 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 68degrees C., 0.2×SSC, 0.1% SDS. SSPE (1×SSPE is 0.15M NaCl, 10 mM NaH.sub.2 PO.sub.4, 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 (Tm) of the hybrid, where Tm is determined according to the following equations. For hybrids less than 18 base pairs in length, Tm (degrees C.)=2(# of A+T bases)+4(# of #G+C bases). For hybrids above 18 base pairs in length, Tm (degrees C.)=81.5+16.6($\log_{10}$ [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.

[0073] The present invention also provides genes corresponding to the nucleic acid sequences disclosed herein. "Corresponding genes" or "corresponding genomic nucleic acids" are the regions of the genome that are transcribed to produce the mRNAs from which cDNA nucleic acid sequences are derived and may include contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes may therefore include but are not limited to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. Corresponding genomic nucleic acids may include 5000 basepairs (more preferably, 2500 basepairs, and most preferably, 1000 basepairs) of genomic nucleic acid sequence upstream of the first nucleotide of the genomic sequence corresponding to the initiation codon of the B7H-1.2 coding sequence, and 5000 basepairs (more preferably, 2500 basepairs, and most preferably, 1000 basepairs) of genomic nucleic acid sequence downstream of the last nucleotide of the genomic sequence corresponding to the termination codon of the B7H-1.2 coding sequence. The corresponding genes or genomic nucleic acids can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. An "isolated gene" or "an isolated genomic nucleic acid" is a genomic nucleic acid that has been separated from the adjacent genomic sequences present in the genome of the organism from which the genomic nucleic acid was isolated.

Methods for Making and Purifying Alpha-Helix-Containing Polypeptides

[0074] Methods for making alpha-helix-containing polypeptides are described below. Expression, isolation, and purification of the polypeptides and fragments of the invention can be accomplished by any suitable technique, including but not limited to the following methods. The isolated nucleic acid of the invention can be-operably linked to an expression control sequence such as the pDC409 vector (Giri et al., 1990, *EMBO J.,* 13: 2821) or the derivative pDC412 vector (Wiley et al., 1995, Immunity 3: 673). The pDC400 series vectors are useful for transient mammalian expression systems, such as CV-1 or 293 cells. Alternatively, the isolated nucleic acid of the invention can be linked to expression vectors such as pDC312, pDC316, or pDC317 vectors. The pDC300 series vectors all contain the SV40 origin of replication, the CMV promoter, the adenovirus tripartite leader, and the SV40 polyA and termination signals, and are useful for stable mammalian expression systems, such as CHO cells or their derivatives. Other expression control sequences and cloning technologies can also be used to produce the polypeptide recombinantly, such as the pMT2 or pED expression vectors (Kaufman et al., 1991, Nucleic Acids Res. 19: 4485-4490; and Pouwels et al., 1985, *Cloning Vectors: A Laboratory Manual,* Elsevier, New York) and the GATEWAY Vectors (lifetech.com/Content/Tech-Online/molecular_biology/manuals_pps/11797016.pdf; Life Technologies; Rockville, Md.). In the GATEWAY system the isolated nucleic acid of the invention, flanked by attB sequences, can be recombined through an integrase reaction with a GATEWAY vector such as pDONR201 containing attP sequences. This provides an entry vector for the GATEWAY system containing the isolated nucleic acid of the invention. This entry vector can be further recombined with other suitably prepared expression control sequences, such as those of the pDC400 and pDC300 series described above. Many suitable expression control sequences are known in the art. General methods of expressing recombinant polypeptides are also described in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As used herein "operably linked" means that the nucleic acid of the invention and an expression control sequence are situated within a construct, vector, or cell in such a way that the polypeptide encoded by the nucleic acid is expressed when appropriate molecules (such as polymerases) are present. As one embodiment of the invention, at least one expression control sequence is operably linked to the nucleic acid of the invention in a recombinant host cell or progeny thereof, the nucleic acid and/or expression control sequence

having been introduced into the host cell by transformation or transfection, for example, or by any other suitable method. As another embodiment of the invention, at least one expression control sequence is integrated into the genome of a recombinant host cell such that it is operably linked to a nucleic acid sequence encoding a polypeptide of the invention. In a further embodiment of the invention, at least one expression control sequence is operably linked to a nucleic acid of the invention through the action of a trans-acting factor such as a transcription factor, either in vitro or in a recombinant host cell.

[0075] In addition, a sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. The choice of signal peptide or leader can depend on factors such as the type of host cells in which the recombinant polypeptide is to be produced. To illustrate, examples of heterologous signal peptides that are functional in mammalian host cells include the signal sequence for interleukin-7 (IL-7) described in U.S. Pat. No. 4,965,195; the signal sequence for interleukin-2 receptor described in Cosman et al., Nature 312:768 (1984); the interleukin-4 receptor signal peptide described in EP 367, 566; the type I interleukin-1 receptor signal peptide described in U.S. Pat. No. 4,968,607; and the type II interleukin-1 receptor signal peptide described in EP 460, 846. A DNA sequence for a signal peptide (secretory leader) can be fused in frame to the nucleic acid sequence of the invention so that the DNA is initially transcribed, and the mRNA translated, into a fusion polypeptide comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the polypeptide. The signal peptide is cleaved from the polypeptide upon secretion of polypeptide from the cell. The skilled artisan will also recognize that the position(s) at which the signal peptide is cleaved can differ from that predicted by computer program, and can vary according to such factors as the type of host cells employed in expressing a recombinant polypeptide. A polypeptide preparation can include a mixture of polypeptide molecules having different N-terminal amino acids, resulting from cleavage of the signal peptide at more than one site.

[0076] Established methods for introducing DNA into mammalian cells have been described (Kaufman, R. J., *Large Scale Mammalian Cell Culture,* 1990, pp. 15-69). Additional protocols using commercially available reagents, such as Lipofectamine lipid reagent (Gibco/BRL) or Lipofectamine-Plus lipid reagent, can be used to transfect cells (Felgner et al., Proc. Natl. Acad. Sci USA 84:7413-7417, 1987). In addition, electroporation can be used to transfect mammalian cells using conventional procedures, such as those in Sambrook et al. (*Molecular Cloning: A Laboratory Manual,* 2 ed. Vol. 1-3, Cold Spring Harbor Laboratory Press, 1989). Selection of stable transformants can be performed using methods known in the art, such as, for example, resistance to cytotoxic drugs. Kaufman et al., *Meth. in Enzymology* 185:487-511, 1990, describes several selection schemes, such as dihydrofolate reductase (DHFR) resistance. A suitable strain for DHFR selection can be CHO strain DX-B 11, which is deficient in DHFR (Urlaub and Chasin, *Proc. Natl. Acad. Sci. USA* 77:4216-4220, 1980). A plasmid expressing the DHFR cDNA can be introduced into strain DX-B 11, and only cells that contain the plasmid can grow in the appropriate selective media. Other examples of selectable markers that can be incorporated into an expression vector include cDNAs conferring resistance to antibiotics, such as G418 and hygromycin B. Cells harboring the vector can be selected on the basis of resistance to these compounds.

[0077] Alternatively, gene products can be obtained via homologous recombination, or "gene targeting," techniques. Such techniques employ the introduction of exogenous transcription control elements (such as the CMV promoter or the like) in a particular predetermined site on the genome, to induce expression of the endogenous nucleic acid sequence of interest (see, for example, U.S. Pat. No. 5,272, 071). The location of integration into a host chromosome or genome can be easily determined by one of skill in the art, given the known location and sequence of the gene. In a preferred embodiment, the present invention also contemplates the introduction of exogenous transcriptional control elements in conjunction with an amplifiable gene, to produce increased amounts of the gene product, again, without the need for isolation of the gene sequence itself from the host cell.

[0078] A number of types of cells may act as suitable host cells for expression of the polypeptide. Mammalian host cells include, for example, the COS-7 line of monkey kidney cells (ATCC CRL 1651) (Gluzman et al., Cell 23:175, 1981), L cells, C127 cells, 3T3 cells (ATCC CCL 163), Chinese hamster ovary (CHO) cells or their derivatives such as Veggie CHO and related cell lines which grow in serum-free media (Rasmussen et al., 1998, *Cytotechnology* 28: 31), HeLa cells, BHK (ATCC CRL 10) cell lines, the CV1/EBNA cell line derived from the African green monkey kidney cell line CV1 (ATCC CCL 70) as described by McMahan et al. (*EMBO J.* 10: 2821, 1991), human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, other transformed primnate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Alternatively, it may be possible to produce the polypeptide in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces* strains, *Candida,* or any yeast strain capable of expressing heterologous polypeptides. Potentially suitable bacterial strains include *Escherichia coli, Bacillus subtilis, Salmonella typhimurium,* or any bacterial strain capable of expressing heterologous polypeptides. If the polypeptide is made in yeast or bacteria, it may be necessary to modify the polypeptide produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional polypeptide. Such covalent attachments may be accomplished using known chemical or enzymatic methods. The polypeptide may also be produced by operably linking the isolated nucleic acid of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, e.g., Invitrogen, San Diego, Calif., U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), and Luckow and Summers, *Bio/Technology* 6:47 (1988). As used herein, an insect cell capable of expressing a nucleic acid of the present invention is "transformed." Cell-free translation systems could also be employed to produce polypeptides using RNAs derived

from nucleic acid constructs disclosed herein. A host cell that comprises an isolated nucleic acid of the invention, preferably operably linked to at least one expression control sequence, is a "recombinant host cell".

[0079] The polypeptide of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant polypeptide. The resulting expressed polypeptide may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the polypeptide may also include an affinity column containing agents which will bind to the polypeptide; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography. Alternatively, the polypeptide of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion polypeptide, such as those of maltose binding polypeptide (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion polypeptides are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and InVitrogen, respectively. The polypeptide can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope (FLAG®) is commercially available from Kodak (New Haven, Conn.). Finally, one or more reverse-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the polypeptide. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant polypeptide. The polypeptide thus purified is substantially free of other mammalian polypeptides and is defined in accordance with the present invention as an "isolated polypeptide"; such isolated polypeptides of the invention include isolated antibodies that bind to alpha-helix-containing polypeptides, fragments, variants, binding partners etc. The polypeptide of the invention may also be expressed as a product of transgenic animals, e.g., as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the polypeptide.

[0080] It is also possible to utilize an affinity column comprising a polypeptide-binding polypeptide of the invention, such as a monoclonal antibody generated against polypeptides of the invention, to affinity-purify expressed polypeptides. These polypeptides can be removed from an affinity column using conventional techniques, e.g., in a high salt elution buffer and then dialyzed into a lower salt buffer for use or by changing pH or other components depending on the affinity matrix utilized, or be competitively removed using the naturally occurring substrate of the affinity moiety, such as a polypeptide derived from the invention. In this aspect of the invention, polypeptide-binding polypeptides, such as the anti-polypeptide antibodies of the invention or other polypeptides that can interact with the polypeptide of the invention, can be bound to a solid phase support such as a column chromatography matrix or a similar substrate suitable for identifying, separating, or purifying cells that express polypeptides of the invention on their surface. Adherence of polypeptide-binding polypeptides of the invention to a solid phase contacting surface can be accomplished by any means, for example, magnetic microspheres can be coated with these polypeptide-binding polypeptides and held in the incubation vessel through a magnetic field. Suspensions of cell mixtures are contacted with the solid phase that has such polypeptide-binding polypeptides thereon. Cells having polypeptides of the invention on their surface bind to the fixed polypeptide-binding polypeptide and unbound cells then are washed away. This affinity-binding method is useful for purifying, screening, or separating such polypeptide-expressing cells from solution. Methods of releasing positively selected cells from the solid phase are known in the art and encompass, for example, the use of enzymes. Such enzymes are preferably non-toxic and non-injurious to the cells and are preferably directed to cleaving the cell-surface binding partner. Alternatively, mixtures of cells suspected of containing polypeptide-expressing cells of the invention first can be incubated with a biotinylated polypeptide-binding polypeptide of the invention. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides the binding of the polypeptide-binding cells to the beads. Use of avidin-coated beads is known in the art. See Berenson, et al. *J. Cell. Biochem.,* 10D:239 (1986). Wash of unbound material and the release of the bound cells is performed using conventional methods.

[0081] The polypeptide may also be produced by known conventional chemical synthesis. Methods for constructing the polypeptides of the present invention by synthetic means are known to those skilled in the art. The. synthetically-constructed polypeptide sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with polypeptides may possess biological properties in common therewith, including polypeptide activity. Thus, they may be employed as biologically active or immunological substitutes for natural, purified polypeptides in screening of therapeutic compounds and in immunological processes for the development of antibodies.

[0082] The desired degree of purity depends on the intended use of the polypeptide. A relatively high degree of purity is desired when the polypeptide is to be administered in vivo, for example. In such a case, the polypeptides are purified such that no polypeptide bands corresponding to other polypeptides are detectable upon analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). It will be recognized by one skilled in the pertinent field that multiple bands corresponding to the polypeptide can be visualized by SDS-PAGE, due to differential glycosylation, differential post-translational processing, and the like. Most preferably, the polypeptide of the invention is purified to substantial homogeneity, as indicated by a single polypeptide band upon analysis by SDS-PAGE. The polypeptide band can be visualized by silver staining, Coomassie blue staining, or (if the polypeptide is radiolabeled) by autoradiography.

Antagonists and Agonists of Alpha-Helix-Containing Polypeptides

[0083] Any method which neutralizes alpha-helix-containing polypeptides or inhibits expression of the alpha-helix-containing genes (either transcription or translation)

can be used to reduce the biological activities of alpha-helix-containing polypeptides. In particular embodiments, antagonists inhibit the binding of at least one alpha-helix-containing polypeptide to cells, thereby inhibiting biological activities induced by the binding of those alpha-helix-containing polypeptides to the cells. In certain other embodiments of the invention, antagonists can be designed to reduce the level of endogenous alpha-helix-containing gene expression, e.g., using well-known antisense or ribozyme approaches to inhibit or prevent translation of alpha-helix-containing mRNA transcripts; triple helix approaches to inhibit transcription of alpha-helix-containing polypeptide genes; or targeted homologous recombination to inactivate or "knock out" the alpha-helix-containing genes or their endogenous promoters or enhancer elements. Such antisense, ribozyme, and triple helix antagonists may be designed to reduce or inhibit either unimpaired, or if appropriate, mutant alpha-helix-containing polypeptide gene activity. Techniques for the production and use of such molecules are well known to those of skill in the art.

[0084] 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 alpha-helix-containing mRNA. The antisense oligonucleotides will bind to the complementary target gene mRNA transcripts and prevent translation. Absolute complementarity, although preferred, is not required. A sequence "complementary" to a portion of a nucleic acid, as referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the nucleic acid, forming a stable duplex (or triplex, as appropriate). In the case of double-stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Preferred 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. However, oligonucleotides complementary to the 5'- or 3'-non-translated, non-coding regions of the alpha-helix-containing polypeptide gene transcript, or to the coding regions, could be used in an antisense approach to inhibit translation of endogenous alpha-helix-containing polypeptide mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA preferably 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. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides. The oligonucleotides can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. Chimeric oligonucleotides, oligonucleosides, or mixed oligonucleotides/oligonucleosides of the invention can be of several different types. These include a first type wherein the "gap" segment of nucleotides is positioned between 5' and 3'"wing" segments of linked nucleosides and a second "open end" type wherein the "gap" segment is located at either the 3' or the 5' terminus of the oligomeric compound (see, e.g., U.S. Pat. No. 5,985,664). Oligonucleotides of the first type are also known in the art as "gapmers" or gapped oligonucleotides. Oligonucleotides of the second type are also known in the art as "hemimers" or "wingmers". The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (se&, 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), or hybridization-triggered cleavage agents or intercalating agents. (See, e.g., Zon, 1988, Pharm. Res. 5: 539-549). The antisense molecules should be delivered to cells which express the alpha-helix-containing polypeptide transcript in vivo. A number of methods have been developed for delivering antisense DNA or RNA to cells; e.g., antisense molecules can be injected directly into the tissue or cell derivation site, or 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. However, it is often difficult to achieve intracellular concentrations of the antisense sufficient to suppress translation of endogenous mRNAs. Therefore a preferred 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 patient will result in the transcription of sufficient amounts of single stranded RNAs that will form complementary base pairs with the enidogenous alpha-helix-containing polypeptide gene transcripts and thereby prevent translation of the alpha-helix-containing polypeptide 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, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells.

[0085] Ribozyme molecules designed to catalytically cleave alpha-helix-containing polypeptide mRNA transcripts can also be used to prevent translation of alpha-helix-containing polypeptide mRNA and expression of alpha-helix-containing polypeptides. (See, e.g., PCT International Publication WO90/11364, published Oct. 4, 1990; U.S. Pat. No. 5,824,519). The ribozymes that can be used in the present invention include hammerhead ribozymes (Haseloff and Gerlach, 1988, Nature, 334:585-591), RNA endoribonucleases (hereinafter "Cech-type ribozymes") such as the one which occurs naturally in Tetrahymena Thermophila (known as the IVS, or L-19 IVS RNA) and which has been extensively described by Thomas Cech and collaborators (International Patent Application No. WO 88/04300; Been and Cech, 1986, Cell, 47:207-216). As in the antisense approach, the ribozymes can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express the alpha-helix-containing polypeptide in vivo. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive pol III or pol II promoter, so that transfected cells will produce suf-

ficient quantities of the ribozyme to destroy endogenous alpha-helix-containing polypeptide messages and inhibit translation. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

[0086] Alternatively, endogenous alpha-helix-containing polypeptide gene expression can be reduced by targeting deoxyribonucleotide sequences complementary to the regulatory region of the target gene (i.e., the target gene promoter and/or enhancers) to form triple helical structures that prevent transcription of the target alpha-helix-containing polypeptide gene. (See generally, Helene, 1991, Anticancer Drug Des., 6(6), 569-584; Helene, et al., 1992, Ann. N.Y. Acad. Sci., 660, 27-36; and Maher, 1992, Bioassays 14(12), 807-815).

[0087] Anti-sense RNA and DNA, ribozyme, and triple helix molecules of the invention may be prepared by any method known in the art for the synthesis of DNA and RNA molecules. These include techniques for chemically synthesizing oligodeoxyribonucleotides and oligoribonucleotides well known in the art such as for example solid phase phosphoramidite chemical synthesis. Oligonucleotides can be synthesized by standard methods known in the art, e.g. by use of an automated DNA synthesizer (such as are commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al., 1988, Nucl. Acids Res. 16:3209. Methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer 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.

[0088] Endogenous target gene expression can also be reduced by inactivating or "knocking out" the target gene or its promoter using targeted homologous recombination (e.g., see Smithies, et al., 1985, Nature 317, 230-234; Thomas and Capecchi, 1987, Cell 51, 503-512; Thompson, et al., 1989, Cell 5, 313-321). For example, a mutant, non-functional target gene (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous target gene (either the coding regions or regulatory regions of the target gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express the target gene in vivo. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the target gene. Such approaches are particularly suited in the agricultural field where modifications to ES (embryonic stem) cells can be used to generate animal offspring with an inactive target gene (e.g., see Thomas and Capecchi, 1987 and Thompson, 1989, supra), or in model organisms such as *Caenorhabditis elegans* where the "RNA interference" ("RNAi") technique (Grishok A, Tabara H, and Mello C C, 2000, Genetic requirements for inheritance of RNAi in *C. elegans*, Science 287 (5462): 2494-2497), or the introduction of transgenes (Dernburg A F, Zalevsky J, Colaiacovo M P, and Villeneuve A M, 2000, Transgene-mediated cosuppression in the *C. elegans* germ line, Genes Dev. 14 (13): 1578-1583) are used to inhibit the expression of specific target genes. However this approach can be adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site in vivo using appropriate vectors such as viral vectors.

[0089] Organisms that have enhanced, reduced, or modified expression of the gene(s) corresponding to the nucleic acid sequences disclosed herein are provided. The desired change in gene expression can be achieved through the use of antisense nucleic acids or ribozymes that bind and/or cleave the mRNA transcribed from the gene (Albert and Morris, 1994, Trends Pharmacol. Sci. 15(7): 250-254; Lavarosky et al., 1997, Biochem. Mol. Med. 62(1): 11-22; and Hampel, 1998, Prog. Nucleic Acid Res. Mol. Biol. 58: 1-39). Transgenic animals that have multiple copies of the gene(s) corresponding to the nucleic acid sequences disclosed herein, preferably produced by transformation of cells with genetic constructs that are stably maintained within the transformed cells and their progeny, are provided. Transgenic animals that have modified genetic control regions that increase or reduce gene expression levels, or that change temporal or spatial patterns of gene expression, are also provided (see European Patent No. 0 649 464 B1). In addition, organisms are provided in which the gene(s) corresponding to the nucleic acid sequences disclosed herein have been partially or completely inactivated, through insertion of extraneous sequences into the corresponding gene(s) or through deletion of all or part of the corresponding gene(s). Partial or complete gene inactivation can be accomplished through insertion, preferably followed by imprecise excision, of transposable elements (Plasterk, 1992, Bioessays 14(9): 629-633; Zwaal et al., 1993, Proc. Natl. Acad. Sci. USA 90(16): 7431-7435; Clark et al., 1994, Proc. Natl. Acad. Sci. USA 91(2): 719-722), or through homologous recombination, preferably detected by positive/negative genetic selection strategies (Mansour et al., 1988, Nature 336: 348-352; U.S. Pat. Nos. 5,464,764; 5,487,992; 5,627,059; 5,631,153; 5,614,396; 5,616,491; and 5,679,523). These organisms with altered gene expression are preferably eukaryotes and more preferably are mammals. Such organisms are useful for the development of non-human models for the study of disorders involving the corresponding gene(s), and for the development of assay systems for the identification of molecules that interact with the polypeptide product(s) of the corresponding gene(s).

[0090] Also encompassed within the invention are alpha-helix-containing polypeptide variants with partner binding sites that have been altered in conformation so that (1) the alpha-helix-containing polypeptide variant will still bind to its partner(s), but a specified small molecule will fit into the altered binding site and block that interaction, or (2) the alpha-helix-containing polypeptide variant will no longer bind to its partner(s) unless a specified small molecule is present (see for example Bishop et al., 2000, *Nature* 407: 395401). Nucleic acids encoding such altered alpha-helix-containing polypeptides can be introduced into organisms according to methods described herein, and may replace the endogenous nucleic acid sequences encoding the corresponding alpha-helix-containing polypeptide. Such methods allow for the interaction of a particular alpha-helix-containing polypeptide with its binding partners to be regulated by

administration of a small molecule compound to an organism, either systemically or in a localized manner.

[0091] The alpha-helix-containing polypeptides themselves can also be employed in inhibiting a biological activity of alpha-helix-containing polypeptide in in vitro or in vivo procedures. Encompassed within the invention are Helix A and Helix D and other domains of alpha-helix-containing polypeptides that act as "dominant negative" inhibitors of native alpha-helix-containing polypeptide function when expressed as components of fusion polypeptides. For example, a purified polypeptide domain of the present invention can be used to inhibit binding of alpha-helix-containing polypeptides to alpha-helix-containing polypeptide receptors or other endogenous binding partners. Such use effectively would block alpha-helix-containing polypeptide interactions and inhibit alpha-helix-containing polypeptide activities. In still another aspect of the invention, a soluble form of the alpha-helix-containing polypeptide binding partner is used to bind to, and competitively inhibit, activation of the endogenous alpha-helix-containing polypeptide. Furthermore, antibodies which bind to alpha-helix-containing polypeptides often inhibit immunomodulatory activity and act as antagonists. For example, antibodies that specifically recognize one or more epitopes of alpha-helix-containing polypeptides, or epitopes of conserved variants of alpha-helix-containing polypeptides, or peptide fragments of the alpha-helix-containing polypeptide can be used in the invention to inhibit immunomodulatory activity. Such 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. Alternatively, purified and modified alpha-helix-containing polypeptides of the present invention can be administered to modulate interactions between alpha-helix-containing polypeptides and alpha-helix-containing polypeptide binding partners that are not membrane-bound. Such an approach will allow an alternative method for the modification of alpha-helix-containing polypeptide-influenced bioactivity.

[0092] In an alternative aspect, the invention further encompasses the use of agonists of immunomodulatory activity to treat or ameliorate the symptoms of a disease for which increased immunomodulatory activity is beneficial. Such diseases include but are not limited to conditions involving the proliferation or the development of cells from pluripotent stem cell precursors. In a preferred aspect, the invention entails administering compositions comprising an alpha-helix-containing polypeptide nucleic acid or an alpha-helix-containing polypeptide to cells in vitro, to cells ex vivo, to cells in vivo, and/or to a multicellular organism such as a vertebrate or mammal. Preferred therapeutic forms of alpha-helix-containing polypeptide are soluble forms, as described above. In still another aspect of the invention, the compositions comprise administering a alpha-helix-containing polypeptide-encoding nucleic acid for expression of a alpha-helix-containing polypeptide in a host organism for treatment of disease. Particularly preferred in this regard is expression in a human patient for treatment of a dysfunction associated with aberrant (e.g., decreased) endogenous activity of a alpha-helix-containing polypeptide. Furthermore, the invention encompasses the administration to cells and/or organisms of compounds found to increase the endogenous activity of alpha-helix-containing polypeptides. One example of compounds that increase immunomodulatory activity are agonistic antibodies, preferably monoclonal antibodies, that bind to alpha-helix-containing polypeptides or binding partners, which may increase immunomodulatory activity by causing constitutive intracellular signaling (or "ligand mimicking"), or by preventing the binding of a native inhibitor of immunomodulatory activity.

Antibodies to Alpha-Helix-Containing Polypeptides

[0093] Antibodies that are immunoreactive with the polypeptides of the invention are provided herein. Such antibodies specifically bind to the polypeptides via the antigen-binding sites of the antibody (as opposed to non-specific binding). In the present invention, specifically binding antibodies are those that will specifically recognize and bind with alpha-helix-containing polypeptides, homologues, and variants, but not with other molecules. In one preferred embodiment, the antibodies are specific for the polypeptides of the present invention and do not cross-react with other polypeptides. In this manner, the alpha-helix-containing polypeptides, fragments, variants, fusion polypeptides, etc., as set forth above can be employed as "immunogens" in producing antibodies immunoreactive therewith.

[0094] More specifically, the polypeptides, fragment, variants, fusion polypeptides, etc. 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 (Janeway and Travers, *Immuno Biology* 3:9 (Garland Publishing Inc., 2nd ed. 1996)). Because folded polypeptides have complex surfaces, the number of epitopes available is quite numerous; however, due to the conformation of the polypeptide and steric hindrances, the number of antibodies that actually bind to the epitopes is less than the number of available epitopes (Janeway and Travers, *Immuno Biology* 2:14 (Garland Publishing Inc., 2nd ed. 1996)). Epitopes can be identified by any of the methods known in the art. Thus, one aspect of the present invention relates to the antigenic epitopes of the polypeptides of the invention. Such epitopes are useful for raising antibodies, in particular monoclonal antibodies, as described in more detail below. 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 well known in the art such as solid-phase synthesis, chemical or enzymatic cleavage of a polypeptide, or using recombinant DNA technology.

[0095] As to the antibodies that can be elicited by the epitopes of the polypeptides of the invention, whether the epitopes have been isolated or remain part of the polypeptides, both polyclonal and monoclonal antibodies 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 (Kozbor et al., 1984, *J. Immunol.* 133:3001-3005; Cole et al., 1983, *Proc. Natl. Acad. Sci. USA* 80:2026-2030); and the EBV-hybridoma technique (Cole et al., 1985, Monoclonal Antibodies And Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Hybridoma cell lines that produce monoclonal antibodies specific for the polypeptides of the invention are also contemplated herein. Such hybridomas can be produced and identified by conventional techniques. The hybridoma producing the mAb of this invention may be cultivated in vitro or in vivo. Production of high titers of mAbs in vivo makes this the presently preferred method of production. One method for producing such a hybridoma cell line comprises immunizing an animal with a polypeptide; harvesting spleen cells from the immunized animal; fusing said spleen cells to a myeloma cell line, thereby generating hybridoma cells; and identifying a hybridoma cell line that produces a monoclonal antibody that binds the polypeptide. For the production of antibodies, various host animals, may be immunized by injection with one or more of the following: a alpha-helix-containing polypeptide, a fragment of a alpha-helix-containing polypeptide, a functional equivalent of a alpha-helix-containing polypeptide, or a mutant form of a alpha-helix-containing polypeptide. Such host animals may include but are not limited to rabbits, mice, and rats. Various adjuvants may be used to increase the immunologic response, depending on the host species, including but 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 adjutants 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.

[0096] In addition, techniques developed for the production of "chimeric antibodies" (Takeda et al., 1985, *Nature,* 314:452-454; Morrison et al., 1984, *Proc Natl Acad Sci USA* 81:6851-6855; Boulianne et al., 1984, *Nature* 312:643646; Neuberger et al., 1985, Nature 314:268-270) 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 present 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. In one embodiment, a humanized monoclonal antibody comprises the variable region of a murine antibody (or just the antigen binding site thereof) and a constant region derived from a human antibody. Alternatively, a humanized antibody fragment can comprise the antigen binding site of a murine monoclonal antibody and a variable region fragment (lacking the antigen-binding site) derived from a human antibody. 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). Useful techniques for humanizing antibodies are also discussed in U.S. Pat. No. 6,054,297. 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. Preferably, for use in humans, the antibodies are human or humanized; techniques for creating such human or humanized antibodies are also well known and are commercially available from, for example, Medarex Inc. (Princeton, N.J.) and Abgenix Inc. (Fremont, Calif.). In another preferred embodiment, fully human antibodies for use in humans are produced by screening a library of human antibody variable domains using either phage display methods (Vaughan et al., 1998, *Nat Biotechnol.* 16(6): 535-539; and U.S. Pat. No. 5,969,108), ribosome display methods (Schaffitzel et al., 1999, *J Immunol Methods* 231(1-2): 119-135), or mRNA display methods (Wilson et al., 2001, *Proc Natl Acad Sci USA* 98(7): 3750-3755).

[0097] Antigen-binding 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., 1989, Science, 246:1275-1281) 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, 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; and Ward et al., 1989, Nature 334:544-546) can also be adapted to produce single chain antibodies against alpha-helix-containing polypeptide gene products. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Such single chain antibodies may also be useful intracellularly (i.e., as 'intrabodies), for example as described by Marasco et al. (*J. Immunol. Methods* 231:223-238, 1999) for genetic therapy in HIV infection. In addition, antibodies to the alpha-helix-containing polypeptide can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" the alpha-helix-containing polypeptide and that may bind to the alpha-helix-containing polypeptide using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, 1993, *FASEB J* 7(5):437-444; and Nissinoff, 1991, *J. Immunol.* 147(8):2429-2438).

[0098] Antibodies that are immunoreactive with the polypeptides of the invention include bispecific antibodies (i.e., antibodies that are immunoreactive with the polypeptides of the invention via a first antigen binding domain, and also immunoreactive with a different polypeptide via a second antigen binding domain). A variety of bispecific antibodies have been prepared, and found useful both in vitro and in vivo (see, for example, U.S. Pat. No. 5,807,706; and Cao and Suresh, 1998, Bioconjugate Chem 9: 635-644). Numerous methods of preparing bispecific antibodies are known in the art, including the use of hybrid-hybridomas such as quadromas, which are formed by fusing two differed hybridomas, and triomas, which are formed by fusing a hybridoma with a lymphocyte (Milstein and Cuello, 1983,

Nature 305: 537-540; U.S. Pat. No. 4,474,893; and U.S. Pat. No. 6,106,833). U.S. Pat. No. 6,060,285 discloses a process for the production of bispecific antibodies in which at least the genes for the light chain and the variable portion of the heavy chain of an antibody having a first specificity are transfected into a hybridoma cell secreting an antibody having a second specificity. Chemical coupling of antibody fragments has also been used to prepare antigen-binding molecules having specificity for two different antigens (Brennan et al., 1985, *Science* 229: 81-83; Glennie et al., *J. Immunol.,* 1987, 139:2367-2375; and U.S. Pat. No. 6,010,902). Bispecific antibodies can also be produced via recombinant means, for example, by using the leucine zipper moieties from the Fos and Jun proteins (which preferentially form heterodimers) as described by Kostelny et al. (*J. Immunol.* 148:15474553; 1992). U.S. Pat. No. 5,582,996 discloses the use of complementary interactive domains (such as leucine zipper moieties or other lock and key interactive domain structures) to facilitate heterodimer formation in the production of bispecific antibodies. Tetravalent, bispecific molecules can be prepared by fusion of DNA encoding the heavy chain of an F(ab')2 fragment of an antibody with either DNA encoding the heavy chain of a second F(ab')2 molecule (in which the CH1 domain is replaced by a CH3 domain), or with DNA encoding a single chain FV fragment of an antibody, as described in U.S. Pat. No. 5,959,083. Expression of the resultant fusion genes in mammalian cells, together with the genes for the corresponding light chains, yields tetravalent bispecific molecules having specificity for selected antigens. Bispecific antibodies can also be produced as described in U.S. Pat. No. 5,807,706. Generally, the method involves introducing a protuberance (constructed by replacing small amino acid side chains with larger side chains) at the interface of a first polypeptide and a corresponding cavity (prepared by replacing large amino acid side chains with smaller ones) in the interface of a second polypeptide. Moreover, single-chain variable fragments (sFvs) have been prepared by covalently joining two variable domains; the resulting antibody fragments can form dimers or trimers, depending on the length of a flexible linker between the two variable domains (Kortt et al., 1997, *Protein Engineering* 10:423-433).

[0099] Screening procedures by which such antibodies can be identified are well known, and can involve immunoaffinity chromatography, for example. Antibodies can be screened for agonistic (i.e., ligand-mimicking) properties. Such antibodies, upon binding to cell surface alpha-helix-containing polypeptide, induce biological effects (e.g., transduction of biological signals) similar to the biological effects induced when the alpha-helix-containing polypeptide binding partner binds to cell surface alpha-helix-containing polypeptide. Agonistic antibodies can be used to induce alpha-helix-containing polypeptide-mediated cell stimulatory pathways or intercellular communication. Bispecific antibodies can be identified by screening with two separate assays, or with an assay wherein the bispecific antibody serves as a bridge between the first antigen and the second antigen (the latter is coupled to a detectable moiety). Bispecific antibodies that bind alpha-helix-containing polypeptides of the invention via a first antigen binding domain will be useful in diagnostic applications and in treating conditions involving the proliferation or the development of cells from pluripotent stem cell precursors.

[0100] Those antibodies that can block binding of the alpha-helix-containing polypeptides of the invention to binding partners for alpha-helix-containing polypeptide can be used to inhibit alpha-helix-containing polypeptide-mediated intercellular communication or cell stimulation that results from such binding. Such blocking antibodies can be identified using any suitable assay procedure, such as by testing antibodies for the ability to inhibit binding of alpha-helix-containing polypeptide binding to certain cells expressing an alpha-helix-containing polypeptide binding partner. Alternatively, blocking antibodies can be identified in assays for the ability to inhibit a biological effect that results from binding of soluble alpha-helix-containing polypeptide to target cells. Antibodies can be assayed for the ability to inhibit alpha-helix-containing polypeptide binding partner-mediated cell stimulatory pathways, for example. Such an antibody can be employed in an in vitro procedure, or administered in vivo to inhibit a biological activity mediated by the entity that generated the antibody. Disorders caused or exacerbated (directly or indirectly) by the interaction of alpha-helix-containing polypeptide with cell surface binding partner receptor thus can be treated. A therapeutic method involves in vivo administration of a blocking antibody to a mammal in an amount effective in inhibiting alpha-helix-containing polypeptide binding partner-mediated biological activity. Monoclonal antibodies are generally preferred for use in such therapeutic methods. In one embodiment, an antigen-binding antibody fragment is employed. Compositions comprising an antibody that is directed against alpha-helix-containing polypeptide, and a physiologically acceptable diluent, excipient, or carrier, are provided herein. Suitable components of such compositions are as described below for compositions containing alpha-helix-containing polypeptides.

[0101] Also provided herein are conjugates comprising a detectable (e.g., diagnostic) or therapeutic agent, attached to the antibody. Examples of such agents are presented above. The conjugates find use in in vitro or in vivo procedures. The antibodies of the invention can also be used in assays to detect the presence of the polypeptides or fragments of the invention, either in vitro or in vivo. The antibodies also can be employed in purifying polypeptides or fragments of the invention by immunoaffinity chromatography.

Assays of Activities of Alpha-Helix-Containing Polypeptides

[0102] The purified alpha-helix-containing polypeptides of the invention (including polypeptides, polypeptides, fragments, variants, oligomers, and other forms) are useful in a variety of assays. For example, the alpha-helix-containing polypeptide molecules of the present invention can be used to identify binding partners of alpha-helix-containing polypeptides, which can also be used to modulate intercellular communication, cell stimulation, or immune cell activity. Alternatively, they can be used to identify non-binding-partner molecules or substances that modulate intercellular communication, cell stimulatory pathways, or immune cell activity.

[0103] Assays to Identify Binding Partners. Polypeptides of the alpha-helix-containing and fragments thereof can be used to identify binding partners. For example, they can be tested for the ability to bind a candidate binding partner in any suitable assay, such as a conventional binding assay. To

illustrate, the alpha-helix-containing polypeptide can be labeled with a detectable reagent (e.g., a radionuclide, chromophore, enzyme that catalyzes a colorimetric or fluorometric reaction, and the like). The labeled polypeptide is contacted with cells expressing the candidate binding partner. The cells then are washed to remove unbound labeled polypeptide, and the presence of cell-bound label is determined by a suitable technique, chosen according to the nature of the label.

[0104] One example of a binding assay procedure is as follows. A recombinant expression vector containing the candidate binding partner cDNA is constructed. CV1-EBNA-1 cells in 10 cm^2 dishes are transfected with this recombinant expression vector. CV-1/EBNA-1 cells (ATCC CRL 10478) constitutively express EBV nuclear antigen-1 driven from the CMV Immediate-early enhancer/promoter. CV1-EBNA-1 was derived from the African Green Monkey kidney cell line CV-1 (ATCC CCL 70), as described by McMahan et al., (*EMBO J.* 10:2821, 1991). The transfected cells are cultured for 24 hours, and the cells in each dish then are split into a 24-well plate. After culturing an additional 48 hours, the transfected cells (about 4×10^4 cells/well) are washed with BM-NFDM, which is binding medium (RPMI 1640 containing 25 mg/ml bovine serum albumin, 2 mg/ml sodium azide, 20 mM Hepes pH 7.2) to which 50 mg/ml nonfat dry milk has been added. The cells then are incubated for 1 hour at 37° C. with various concentrations of, for example, a soluble polypeptide/Fc fusion polypeptide made as set forth above. Cells then are washed and incubated with a constant saturating concentration of a ^{125}I-mouse anti-human IgG in binding medium, with gentle agitation for 1 hour at 37° C. After extensive washing, cells are released via trypsinization. The mouse anti-human IgG employed above is directed against the Fc region of human IgG and can be obtained from Jackson Immunoresearch Laboratories, Inc., West Grove, Pa. The antibody is radioiodinated using the standard chloramine-T method. The antibody will bind to the Fc portion of any polypeptide/Fc polypeptide that has bound to the cells. In all assays, non-specific binding of ^{125}I-antibody is assayed in the absence of the Fc fusion polypeptide/Fc, as well as in the presence of the Fc fusion polypeptide and a 200-fold molar excess of unlabeled mouse anti-human IgG antibody. Cell-bound ^{125}I-antibody is quantified on a Packard Autogamma counter. Affinity calculations (Scatchard, *Ann. N.Y. Acad. Sci.* 51:660, 1949) are generated on RS/1 (BBN Software, Boston, Mass.) run on a Microvax computer. Binding can also be detected 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), 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 AB (Uppsala, Sweden). Compounds that can be assayed for binding to alpha-helix-containing polypeptides include but are not limited to small organic molecules, such as those that are comerically 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 assyas are usually less than 10K molecular weight and may possess a number of physicochemical 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 alpha-helix-containing polypeptides.

[0105] Yeast Two-Hybrid or "Interaction Trap" Assays. Where the alpha-helix-containing polypeptide binds or potentially binds to another polypeptide (such as, for example, in a receptor-ligand interaction), the nucleic acid encoding the alpha-helix-containing polypeptide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify nucleic acids encoding the other polypeptide with which binding occurs or to identify inhibitors of the binding interaction. Polypeptides involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

[0106] Competitive Binding Assays. Another type of suitable binding assay is a competitive binding assay. To illustrate, biological activity of a variant can be determined by assaying for the variant's ability to compete with the native polypeptide for binding to the candidate binding partner. Competitive binding assays can be performed by conventional methodology. Reagents that can be employed in competitive binding assays include radiolabeled alpha-helix-containing polypeptide and intact cells expressing alpha-helix-containing polypeptide receptors (endogenous or recombinant) on the cell surface. For example, a radiolabeled soluble alpha-helix-containing polypeptide fragment can be used to compete with a soluble alpha-helix-containing polypeptide variant for binding to cell surface receptors. Instead of intact cells, one could substitute a soluble binding partner/Fc fusion polypeptide bound to a solid phase through the interaction of Polypeptide A or Polypeptide G (on the solid phase) with the Fc moiety. Chromatography columns that contain Polypeptide A and Polypeptide G include those available from Pharmacia Biotech, Inc., Piscataway, N.J.

[0107] Assays to Identify Modulators of Intercellular Communication, Cell Stimulation, or Immune Cell Activity. The influence of alpha-helix-containing polypeptide on intercellular communication, cell stimulation, or immune cell activity can be manipulated to control these activities in target cells. For example, the disclosed alpha-helix-containing polypeptides, nucleic acids encoding the disclosed alpha-helix-containing polypeptides, or agonists or antagonists of such polypeptides can be administered to a cell or group of cells to induce, enhance, suppress, or arrest cellular communication, cell stimulation, or activity in the target cells. Identification of alpha-helix-containing polypeptides, agonists or antagonists that can be used in this manner can be carried out via a variety of assays known to those skilled in the art. Included in such assays are those that evaluate the ability of an alpha-helix-containing polypeptide to influence intercellular communication, cell stimulation or activity. Such an assay would involve, for example, the analysis of

immune cell interaction in the presence of an alpha-helix-containing polypeptide. In such an assay, one would determine a rate of communication or cell stimulation in the presence of the alpha-helix-containing polypeptide and then determine if such communication or cell stimulation is altered in the presence of a candidate agonist or antagonist or another alpha-helix-containing polypeptide. 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.

[0108] In another aspect, the present invention provides a method of detecting the ability of a test compound to affect the intercellular communication or cell stimulatory activity of a cell. In this aspect, the method comprises: (1) contacting a first group of target cells with a test compound including an alpha-helix-containing polypeptide receptor polypeptide or fragment thereof under conditions appropriate to the particular assay being used; (2) measuring the net rate of intercellular communication or cell stimulation among the target cells; and (3) observing the net rate of intercellular communication or cell stimulation among control cells containing the alpha-helix-containing polypeptide receptor polypeptides or fragments thereof, in the absence of a test compound, under otherwise identical conditions as the first group of cells. In this embodiment, the net rate of intercellular communication or cell stimulation in the control cells is compared to that of the cells treated with both the alpha-helix-containing polypeptide molecule as well as a test compound. The comparison will provide a difference in the net rate of intercellular communication or cell stimulation such that an effector of intercellular communication or cell stimulation can be identified. The test compound can function as an effector by either activating or up-regulating, or by inhibiting or down-regulating intercellular communication or cell stimulation, and can be detected through this method.

[0109] Cell Proliferation, Cell Death, Cell Differentiation, and Cell Adhesion Assays. A polypeptide of the present invention may exhibit 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 a polypeptide of the present invention is evidenced by any one of a number of routine factor-dependent cell proliferation assays for cell lines including, without limitation, 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. The activity of a alpha-helix-containing polypeptide of the invention may, among other means, be measured by the following methods:

[0110] 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. 31-3.19: 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.

[0111] 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.

[0112] Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells include, without limitation, those described in: Bottomly et al., 1991, Measurement 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.

[0113] 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., Proc Natl Acad Sci USA 77: 6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988

[0114] 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., Proc. Natl. Acad. Sci. 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., Proc. Natl. Acad. Sci. 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; Bowmanet 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.

[0115] 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.

[0116] 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.

[0117] 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.

[0118] 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.

[0119] 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., Proc Natl Acad Sci. USA 88:7548-7551, 1991

[0120] 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.

[0121] Assays for stem cell survival and differentiation (which will identify, among others, polypeptides that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, 1994, In *Culture of Hematopoietic Cells,* Freshney et al. eds. pp. 265-268, Wiley-Liss, Inc., New York, N.Y.; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece and Briddell, 1994, In *Culture of Hematopoietic Cells,* Freshney et al. eds. pp. 23-39, Wiley-Liss, Inc., New York, N.Y.; Neben et al., Experimental Hematology 22:353-359, 1994; Ploemacher, 1994, Cobblestone area forming cell assay, In *Culture of Hematopoietic Cells,* Freshney et al. eds. pp. 1-21, Wiley-Liss, Inc., New York, N.Y.; Spooncer et al., 1994, Long term bone marrow cultures in the presence of stromal cells, In *Culture of Hematopoietic Cells,* Freshney et al. eds. pp. 163-179, Wiley-Liss, Inc., New York, N.Y.; Sutherland, 1994, Long term culture initiating cell assay, In *Culture of Hematopoietic Cells,* Freshney et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y.

[0122] Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No. WO91/07491 (skin, endothelium). Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach and Rovee, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

[0123] 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 cytokines 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

[0124] 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., Proc. Natl. Acad. Sci. 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.

Rational Design of Compounds that Interact with Alpha-Helix-Containing Polypeptides

[0125] The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact, e.g., inhibitors, agonists, antagonists, etc. Any of these examples can be used to fashion drugs which are more active or stable forms of the polypeptide or which enhance or interfere with the function of a polypeptide in vivo (Hodgson J (1991) Biotechnology 9:19-21). In one approach, the three-dimensional structure of a polypeptide of interest, or of a polypeptide-inhibitor complex, is determined by x-ray crystallography, by nuclear magnetic resonance, or by computer homology modeling or, most typically, by a combination of these approaches. Both the shape and charges of the polypeptide must be ascertained to elucidate the structure and to determine active site(s) of the molecule. Less often, useful information regarding the structure of a polypeptide

may be gained by modeling based on the structure of homologous polypeptides. In both cases, relevant structural information is used to design analogous serpin-like molecules, to identify efficient inhibitors, or to identify small molecules that may bind serpins. Useful examples of rational drug design may include molecules which have improved activity or stability as shown by Braxton S and Wells J A (1992 Biochemistry 31:7796-7801) or which act as inhibitors, agonists, or antagonists of native peptides as shown by Athauda SB et al (1993 J Biochem 113:742-746). The use of alpha-helix-containing polypeptide structural information in molecular modeling software systems to assist in inhibitor design and inhibitor-alpha-helix-containing polypeptide interaction is also encompassed by the invention. A particular method of the invention comprises analyzing the three dimensional structure of alpha-helix-containing polypeptides for likely binding sites of substrates, synthesizing a new molecule that incorporates a predictive reactive site, and assaying the new molecule as described further herein.

[0126] It is also possible to isolate a target-specific antibody, selected by functional assay, as described further herein, and then to solve its crystal structure. This approach, in principle, yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass polypeptide crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced peptides. The isolated peptides would then act as the pharmacore.

Diagnostic and Other Uses of Alpha-Helix-Containing Polypeptides and Nucleic Acids

[0127] The nucleic acids encoding the alpha-helix-containing polypeptides provided by the present invention can be used for numerous diagnostic or other useful purposes. The nucleic acids of the invention can be used to express recombinant polypeptide for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding polypeptide is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel nucleic acids; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-polypeptide antibodies using DNA immunization techniques; as an antigen to raise anti-DNA antibodies or elicit another immune response, and for gene therapy. Uses of alpha-helix-containing polypeptides and fragmented polypeptides include, but are not limited to, the following: purifying polypeptides and measuring the activity thereof; delivery agents; therapeutic and research reagents; molecular weight and isoelectric focusing markers; controls for peptide fragmentation; identification of unknown polypeptides; and preparation of antibodies. Any or all nucleic acids suitable for these uses are capable of being developed into reagent grade or kit format for commercialization as products. Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E. F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S. L. and A. R. Kimmel eds., 1987

[0128] Probes and Primers. Among the uses of the disclosed alpha-helix-containing polypeptide nucleic acids, and combinations of fragments thereof, is the use of fragments as probes or primers. Such fragments generally comprise at least about 17 contiguous nucleotides of a DNA sequence. In other embodiments, a DNA fragment comprises at least 30, or at least 60, contiguous nucleotides of a DNA sequence. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by Sambrook et al., 1989 and are described in detail above. Using knowledge of the genetic code in combination with the amino acid sequences set forth above, sets of degenerate oligonucleotides can be prepared. Such oligonucleotides are useful as primers, e.g., in polymerase chain reactions (PCR), whereby DNA fragments are isolated and amplified. In certain embodiments, degenerate primers can be used as probes for non-human genetic libraries. Such libraries would include but are not limited to cDNA libraries, genomic libraries, and even electronic EST (express sequence tag) or DNA libraries. Homologous sequences identified by this method would then be used as probes to identify non-human alpha-helix-containing polypeptide homologues.

[0129] Chromosome Mapping. The nucleic acids encoding alpha-helix-containing polypeptides, and the disclosed fragments and combinations of these nucleic acids, can be used by those skilled in the art using well-known techniques to identify the human chromosome to which these nucleic acids map. Useful techniques include, but are not limited to, using the sequence or portions, including oligonucleotides, as a probe in various well-known techniques such as radiation hybrid mapping (high resolution), in situ hybridization to chromosome spreads (moderate resolution), and Southern blot hybridization to hybrid cell lines containing individual human chromosomes (low resolution). For example, chromosomes can be mapped by radiation hybridization. PCR is performed using the Whitehead Institute/MIT Center for Genome Research Genebridge4 panel of 93 radiation hybrids, using primers that lie within a putative exon of the gene of interest and which amplify a product from human genomic DNA, but do not amplify hamster genomic DNA. The PCR results are converted into a data vector that is submitted to the Whitehead/MIT Radiation Mapping site (www-seq.wi.mit.edu). The data is scored and the chromosomal assignment and placement relative to known Sequence Tag Site (STS) markers on the radiation hybrid map is provided. Alternatively, the genomic sequences corresponding to nucleic acids encoding a alpha-helix-containing polypeptide are mapped by comparison to sequences in public and proprietary databases, such as the GenBank non-redundant database (ncbi.nlm.nih.gov/BLAST), Locuslink (ncbi.nlm.nih.gov:80/LocusLink/), Online Mendelian Inheritance in Man (OMIM) (ncbi.nlm.nih.gov/Omim),

Gene Map Viewer (ncbi.nlm.nih.gov/genemap), Unigene (ncbi.nlm.nih.gov/cgi-bin/UniGene), AceView (ncbi.nlm.nih.gov/AceView), and proprietary databases such as the Celera Discovery System (celera.com). These computer analyses of available genomic sequence information can provide the identification of the specific chromosomal location of human genomic sequences corresponding to sequences encoding human alpha-helix-containing polypeptides, and the unique genetic mapping relationships between the alpha-helix-containing polypeptide genomic sequences and the genetic map locations of known human genetic disorders.

[0130] Diagnostics and Gene Therapy. The nucleic acids encoding alpha-helix-containing polypeptides, and the disclosed fragments and combinations of these nucleic acids can be used by one skilled in the art using well-known techniques to analyze abnormalities associated with the genes corresponding to these polypeptides. This enables one to distinguish conditions in which this marker is rearranged or deleted. In addition, nucleic acids of the invention or a fragment thereof can be used as a positional marker to map other genes of unknown location. The DNA can be used in developing treatments for any disorder mediated (directly or indirectly) by defective, or insufficient amounts of, the genes corresponding to the nucleic acids of the invention. Disclosure herein of native nucleotide sequences permits the detection of defective genes, and the replacement thereof with normal genes. Defective genes can be detected in in vitro diagnostic assays, and by comparison of a native nucleotide sequence disclosed herein with that of a gene derived from a person suspected of harboring a defect in this gene.

[0131] Methods of Screening for Binding Partners. The alpha-helix-containing polypeptides of the invention each can be used as reagents in methods to screen for or identify binding partners. For example, the alpha-helix-containing polypeptides can be attached to a solid support material and may bind to their binding partners in a manner similar to affinity chromatography. In particular embodiments, a polypeptide is attached to a solid support by conventional procedures. As one example, chromatography columns containing functional groups that will react with functional groups on amino acid side chains of polypeptides are available (Pharmacia Biotech, Inc., Piscataway, N.J.). In an alternative, a polypeptide/Fc polypeptide (as discussed above) is attached to protein A- or protein G-containing chromatography columns through interaction with the Fc moiety. The alpha-helix-containing polypeptides also find use in identifying cells that express a binding partner on the cell surface. Polypeptides are bound to a solid phase such as a column chromatography matrix or a similar suitable substrate. For example, magnetic microspheres can be coated with the polypeptides and held in an incubation vessel through a magnetic field. Suspensions of cell mixtures containing potential binding-partner-expressing cells are contacted with the solid phase having the polypeptides thereon. Cells expressing the binding partner on the cell surface bind to the fixed polypeptides, and unbound cells are washed away. Alternatively, alpha-helix-containing polypeptides can be conjugated to a detectable moiety, then incubated with cells to be tested for binding partner expression. After incubation, unbound labeled matter is removed and the presence or absence of the detectable moiety on the cells is determined. In a further alternative, mixtures of cells suspected of expressing the binding partner are incubated with biotinylated polypeptides. Incubation periods are typically at least one hour in duration to ensure sufficient binding. The resulting mixture then is passed through a column packed with avidin-coated beads, whereby the high affinity of biotin for avidin provides binding of the desired cells to the beads. Procedures for using avidin-coated beads are known (see Berenson, et al. *J. Cell. Biochem.,* 10D:239, 1986). Washing to remove unbound material, and the release of the bound cells, are performed using conventional methods. In some instances, the above methods for screening for or identifying binding partners may also be used or modified to isolate or purify such binding partner molecules or cells expressing them.

[0132] Measuring Biological Activity. Polypeptides also find use in measuring the biological activity of alpha-helix-containing-binding polypeptides in terms of their binding affinity. The polypeptides thus can be employed by those conducting "quality assurance" studies, e.g., to monitor shelf life and stability of polypeptide under different conditions. For example, the polypeptides can be employed in a binding affinity study to measure the biological activity of a binding partner polypeptide that has been stored at different temperatures, or produced in different cell types. The polypeptides also can be used to determine whether biological activity is retained after modification of a binding partner polypeptide (e.g., chemical modification, truncation, mutation, etc.). The binding affinity of the modified polypeptide is compared to that of an unmodified binding polypeptide to detect any adverse impact of the modifications on biological activity of the binding polypeptide. The biological activity of a binding polypeptide thus can be ascertained before it is used in a research study, for example.

[0133] Carriers and Delivery Agents. The polypeptides also find use as carriers for delivering agents attached thereto to cells bearing identified binding partners. The polypeptides thus can be used to deliver diagnostic or therapeutic agents to such cells (or to other cell types found to express binding partners on the cell surface) in in vitro or in vivo procedures. Detectable (diagnostic) and therapeutic agents that can be attached to a polypeptide include, but are not limited to, toxins, other cytotoxic agents, drugs, radionuclides, chromophores, enzymes that catalyze a colorimetric or fluorometric reaction, and the like, with the particular agent being chosen according to the intended application. Among the toxins are ricin, abrin, diphtheria toxin, *Pseudomonas aeruginosa* exotoxin A, ribosomal inactivating polypeptides, mycotoxins such as trichothecenes, and derivatives and fragments (e.g., single chains) thereof. Radionuclides suitable for diagnostic use include, but are not limited to, ^{123}I, ^{131}I, ^{99m}Tc, ^{111}In, and ^{76}Br. Examples of radionuclides suitable for therapeutic use are ^{131}I, ^{211}At, ^{77}Br, ^{186}Re, ^{188}Re, ^{212}Pb, ^{212}Bi, 109Pd, ^{64}Cu, and ^{67}Cu. Such agents can be attached to the polypeptide by any suitable conventional procedure. The polypeptide comprises functional groups on amino acid side chains that can be reacted with functional groups on a desired agent to form covalent bonds, for example. Alternatively, the polypeptide or agent can be derivatized to generate or attach a desired reactive functional group. The derivatization can involve attachment of one of the bifunctional coupling reagents available for attaching various molecules to polypeptides (Pierce Chemical Company, Rockford, Ill.). A number of techniques for radiolabeling polypeptides are known. Radio-

nuclide metals can be attached to polypeptides by using a suitable bifunctional chelating agent, for example. Conjugates comprising polypeptides and a suitable diagnostic or therapeutic agent (preferably covalently linked) are thus prepared. The conjugates are administered or otherwise employed in an amount appropriate for the particular application.

Treating Diseases with Alpha-Helix-Containing Polypeptides and Antagonists Thereof

[0134] The alpha-helix-containing polypeptides, fragments, variants, antagonists, agonists, antibodies, and binding partners of the invention are likely to be useful for treating medical conditions and diseases including, but not limited to, conditions and diseases involving the proliferation or the development of cells from pluripotent stem cell precursors. The therapeutic molecule or molecules to be used will depend on the etiology of the condition to be treated and the biological pathways involved, and variants, fragments, and binding partners of alpha-helix-containing polypeptides may have effects similar to or different from alpha-helix-containing polypeptides. For example, an antagonist of the stimulation of cell proliferation activity of alpha-helix-containing polypeptides can be selected for treatment of conditions involving excess proliferation and/or differentiation of cells from pluripotent stem cell precursors, but a particular fragment of a given alpha-helix-containing polypeptide may also act as an effective dominant negative antagonist of that activity. Therefore, in the following paragraphs "alpha-helix-containing polypeptides" refers to all alpha-helix-containing polypeptides, fragments, variants, agonists, antibodies, and binding partners etc. of the invention having or increasing immunomodulatory activity, and "alpha-helix-containing polypeptide antagonists" refers to all alpha-helix-containing polypeptide fragments, variants, antagonists, antibodies, and binding partners etc. of the invention that antagonize immunomodulatory activity, and it is understood that a specific molecule or molecules can be selected from those provided as embodiments of the invention by individuals of skill in the art, according to the biological and therapeutic considerations described herein.

[0135] The disclosed alpha-helix-containing polypeptides, compositions and combination therapies described herein are useful in medicines for treating bacterial, viral, or protozoal infections, and complications resulting therefrom. One such disease is Mycoplasma pneumonia. In addition, provided herein is the use of alpha-helix-containing polypeptides to treat infection by the AIDS virus and thus to prevent or ameliorate related conditions, such as AIDS dementia complex, AIDS associated wasting, lipidistrophy due to antiretroviral therapy; and Kaposi's sarcoma. Provided herein is the use of alpha-helix-containing polypeptides for treating protozoal diseases, including malaria and schistosomiasis. Additionally provided is the use of alpha-helix-containing polypeptides to treat erythema nodosum leprosum; bacterial or viral meningitis; tuberculosis, including pulmonary tuberculosis; and pneumonitis secondary to a bacterial or viral infection. Provided also herein is the use of alpha-helix-containing polypeptides to prepare medicaments for treating louse-borne relapsing fevers, such as that caused by *Borrelia recurrentis*. The alpha-helix-containing polypeptides of the invention can also be used to prepare a medicament for treating conditions caused by Herpes viruses, such as herpetic stromal keratitis, corneal lesions, and virus-induced corneal disorders. In addition, alpha-helix-containing polypeptides can be used in treating human papillomavirus infections. The alpha-helix-containing polypeptides of the invention are used also to prepare medicaments to treat influenza.

[0136] In addition, the disclosed alpha-helix-containing polypeptides, compositions and combination therapies can be used to treat anemias and hematologic disorders, including anemia of chronic disease, autoimmune hemolytic anemia, aplastic anemia, including Fanconi's aplastic anemia; idiopathic thrombocytopenic purpura (ITP); and myelodysplastic syndromes (including refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation).

[0137] Also, the disclosed alpha-helix-containing polypeptides, compositions and combination therapies are useful for treating obesity, including treatment to bring about an increase in leptin formation or binding to leptin receptors in the brain. The alpha-helix-containing polypeptides of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth; infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; suppressing bodily characteristics, including, without limitation, weight or fat to lean ratio; increasing the metabolism, catabolism, processing, utilization, or elimination of dietary fat or lipid; suppressing behavioral characteristics, including, without limitation, appetite; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; and the ability to act as an adjuvant in a vaccine composition.

[0138] Alpha-helix-containing polypeptide antagonists are useful in the treatment of disorders involving inflammation and/or excess cell proliferation. For example, certain cardiovascular disorders are treatable with the disclosed alpha-helix-containing polypeptide antagonists, pharmaceutical compositions or combination therapies, including chronic autoimmune myocarditis and viral myocarditis; and chronic heart failure (CHF). Provided also are methods for using alpha-helix-containing polypeptide antagonists, compositions or combination therapies to treat certain disorders of the endocrine system. For example, the alpha-helix-containing polypeptides or antagonists are used to treat autoimmune types of diabetes and Hashimoto's thyroiditis (i.e., autoimmune thyroiditis). In addition, the disclosed alpha-helix-containing polypeptide antagonists, compositions and combination therapies are used to treat various disorders that involve hearing loss such as inner ear or cochlear nerve-associated hearing loss that is thought to result from an autoimmune process, i.e., autoimmune hearing loss. This condition currently is treated with steroids, methotrexate and/or cyclophosphamide, which may be administered concurrently with the alpha-helix-containing polypeptide antagonists. Certain conditions of the gastrointestinal system also are treatable with alpha-helix-containing polypeptide antagonists, compositions or combination therapies, including Crohn's disease and ulcerative colitis. The disclosed alpha-helix-containing polypeptide antagonists, compositions and combination therapies are further used to treat conditions of the liver such as inflammation of the liver due to unknown causes. A number of pulmonary disorders also can be treated with the disclosed

alpha-helix-containing polypeptide antagonists, compositions and combination therapies, such as allergies, including allergic rhinitis, contact dermatitis, atopic dermatitis, and asthma. Disorders involving the skin or mucous membranes also are treatable using the disclosed alpha-helix-containing polypeptides or antagonists, compositions or combination therapies. Such disorders include inflammatory skin diseases and hyperproliferative disorders such as, for example, psoriasis. Disorders associated with transplantation also are treatable with the disclosed alpha-helix-containing polypeptide antagonists, compositions or combination therapies, such as graft-versus-host disease, and complications resulting from solid organ transplantation, including transplantion of heart, liver, lung, skin, kidney or other organs. Alpha-helix-containing polypeptide antagonists may be administered, for example, to prevent or inhibit the development of bronchiolitis obliterans after lung transplantation. Certain ocular disorders also are treatable with the disclosed alpha-helix-containing polypeptide antagonists, compositions or combination therapies, including inflammatory eye disease, inflammatory eye disease associated with smoking, and macular degeneration. Also, the alpha-helix-containing polypeptide antagonists, compositions and combination therapies of the invention are used to suppress the inflammatory response prior, during or after the transfusion of allogeneic red blood cells in cardiac or other surgery, or in treating a traumatic injury to a limb or joint, such as traumatic knee injury.

[0139] Also provided herein are methods for using alpha-helix-containing polypeptide antagonists, compositions or combination therapies to treat various hematologic and oncologic disorders. For example, alpha-helix-containing polypeptide antagonists are used to treat various forms of cancer, including acute myelogenous leukemia, Epstein-Barr virus-positive nasopharyngeal carcinoma, glioma, colon, stomach, prostate, renal cell, cervical and ovarian cancers, lung cancer (SCLC and NSCLC), including cancer-associated cachexia, fatigue, asthenia, paraneoplastic syndrome of cachexia and hypercalcemia. Additional diseases treatable with the subject alpha-helix-containing polypeptide antagonists, compositions or combination therapies are solid tumors, including sarcoma, osteosarcoma, and carcinoma, such as adenocarcinoma (for example, breast cancer) and squamous cell carcinoma. In addition, the subject alpha-helix-containing polypeptide antagonists, compositions or combination therapies are useful for treating leukemia, including acute Tyelogenous leukemia, chronic or acute lymphoblastic leukemia and hairy cell leukemia. Other malignancies with invasive metastatic potential can be treated with the subject alpha-helix-containing polypeptide antagonists, compositions and combination therapies, including multiple myeloma. Various lymphoproliferative disorders also are treatable with the disclosed alpha-helix-containing polypeptide antagonists, compositions or combination therapies. These include, but are not limited to autoimmune lymphoproliferative syndrome (ALPS), chronic lymphoblastic leukemia, hairy cell leukemia, chronic lymphatic leukemia, peripheral T-cell lymphoma, small lymphocytic lymphoma, mantle cell lymphoma, follicular lymphoma, Burkitt's lymphoma, Epstein-Barr virus-positive T cell lymphoma, histiocytic lymphoma, Hodgkin's disease, diffuse aggressive lymphoma, acute lymphatic leukemias, T gamma lymphoproliferative disease, cutaneous B cell lymphoma, cutaneous T cell lymphoma (i.e., mycosis fungoides) and Sézary syndrome.

Administration of Alpha-Helix-Containing Polypeptides and Antagonists Thereof

[0140] This invention provides compounds, compositions, and methods for treating a patient, preferably a mammalian patient, and most preferably a human patient, who is suffering from a medical disorder, and in particular a alpha-helix-containing polypeptide-mediated disorder. Such alpha-helix-containing polypeptide-mediated disorders include conditions caused (directly or indirectly) or exacerbated by binding between alpha-helix-containing polypeptide and a binding partner. For purposes of this disclosure, the terms "illness,""disease,""medical condition,""abnormal condition" and the like are used interchangeably with the term "medical disorder." The terms "treat", "treating", and "treatment" used herein includes curative, preventative (e.g., prophylactic) and palliative or ameliorative treatment. For such therapeutic uses, alpha-helix-containing polypeptides and fragments, alpha-helix-containing polypeptide nucleic acids encoding the alpha-helix-containing polypeptides, and/or agonists or antagonists of the alpha-helix-containing polypeptide such as antibodies can be administered to the patient in need through well-known means. Compositions of the present invention can contain a polypeptide in any form described herein, such as native polypeptides, variants, derivatives, oligomers, and biologically active fragments. In particular embodiments, the composition comprises a soluble polypeptide or an oligomer comprising soluble alpha-helix-containing polypeptides.

[0141] Therapeutically Effective Amount. In practicing the method of treatment or use of the present invention, a therapeutically effective amount of a therapeutic agent of the present invention is administered to a patient having a condition to be treated, preferably to treat or ameliorate diseases associated with the activity of a alpha-helix-containing polypeptide. "Therapeutic agent" includes without limitation any of the alpha-helix-containing polypeptides, fragments, and variants; nucleic acids encoding the alpha-helix-containing polypeptides, fragments, and variants; agonists or antagonists of the alpha-helix-containing polypeptides such as antibodies; alpha-helix-containing polypeptide binding partners; complexes formed from the alpha-helix-containing polypeptides, fragments, variants, and binding partners, etc. As used herein, the term "therapeutically effective amount" means the total amount of each therapeutic agent or other active component of the pharmaceutical composition or method 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 agent or active ingredient, 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 agent 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 weeks. The degree of improvement is determined based on signs or symptoms, and determinations may 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 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 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 agents such as alpha-helix-containing polypeptides or antagonists 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. For injuries or acute conditions, a single dose may be sufficient. 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.

[0142] Dosing. 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 may be formulated in, animal models to achieve a circulating plasma concentration range that includes the IC50 (ie., 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 may 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 pharmaceutical 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, alpha-helix-containing polypeptides or antagonists 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 alpha-helix-containing polypeptides or antagonists per adult dose ranges from 1-20 mg/m^2, and preferably is about 5-12 mg/m^2. Alternatively, a flat dose may 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 alpha-helix-containing polypeptides or antagonists at 25 mg/dose, or alternatively, containing 50 mg per dose. The 25 mg or 50 mg dose may 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 alpha-helix-containing polypeptides or antagonists one to three times per week over a period of at least three weeks, or a dose of 50 mg of alpha-helix-containing polypeptides or antagonists 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 may 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 alpha-helix-containing polypeptides or antagonists, administered by subcutaneous injection one or more times per week. If an antibody against a alpha-helix-containing polypeptide is used as the alpha-helix-containing polypeptide antagonist, a preferred dose range is 0.1 to 20 mg/kg, and more preferably is 1-10 mg/kg. Another preferred dose range for an anti-alpha-helix-containing polypeptide antibody 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 may be injected or administered intravenously.

[0143] Formulations. Compositions comprising an effective amount of a alpha-helix-containing polypeptide of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources), in combination with other components such as a physiologically acceptable diluent, carrier, or excipient, are provided herein. 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). Formulations suitable for administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The polypeptides can be formulated according to known

methods used to prepare pharmaceutically useful compositions. They can be combined in admixture, either as the sole active material or with other known active materials suitable for a given indication, with pharmaceutically acceptable diluents (e.g., saline, Tris-HCl, acetate, and phosphate buffered solutions), preservatives (e.g., thimerosal, benzyl alcohol, parabens), emulsifiers, solubilizers, adjuvants and/or carriers. Suitable formulations for pharmaceutical compositions include those described in *Remington's Pharmaceutical Sciences,* 16th ed. 1980, Mack Publishing Company, Easton, Pa. In addition, such compositions can be complexed with polyethylene glycol (PEG), metal ions, or incorporated into polymeric compounds such as polyacetic acid, polyglycolic acid, hydrogels, dextran, etc., or incorporated into liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts or spheroblasts. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Pat. No. 4,235,871; U.S. Pat. No. 4,501,728; U.S. Pat. No. 4,837,028; and U.S. Pat. No. 4,737,323. Such compositions will influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance, and are thus chosen according to the intended application, so that the characteristics of the carrier will depend on the selected route of administration. In one preferred embodiment of the invention, sustained-release forms of alpha-helix-containing polypeptides are used. Sustained-release forms suitable for use in the disclosed methods include, but are not limited to, alpha-helix-containing polypeptides that are encapsulated in a slowly-dissolving biocompatible polymer (such as the alginate microparticles described in U.S. No. 6,036,978), admixed with such a polymer (including topically applied hydrogels), and or encased in a biocompatible semi-permeable implant.

[0144] Combinations of Therapeutic Compounds. A alpha-helix-containing polypeptide of the present invention may be active in multimers (e.g., heterodimers or homodimers) or complexes with itself or other polypeptides. As a result, pharmaceutical compositions of the invention may comprise a polypeptide of the invention in such multimeric or complexed form. The pharmaceutical composition of the invention may be in the form of a complex of the polypeptide(s) of present invention along with polypeptide or peptide antigens. The invention further includes the administration of alpha-helix-containing polypeptides or antagonists concurrently with one or more other drugs that are administered to the same patient in combination with the alpha-helix-containing polypeptides or antagonists, 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 may be administered in the same or in separate compositions, and by the same or different routes of administration. Examples of components that may be included in the pharmaceutical composition of the invention are: cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, 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, IL-17, IL-18, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. The pharmaceutical composition may 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, a alpha-helix-containing polypeptide or antagonist of the present invention may be included in formulations of the particular alpha-helix-containing polypeptide, 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. Additional examples 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. Additionally, alpha-helix-containing polypeptides or antagonists may be combined with a second alpha-helix-containing polypeptide/antagonist, including an antibody against a alpha-helix-containing polypeptide, or a alpha-helix-containing polypeptide-derived peptide that acts as a competitive inhibitor of a native alpha-helix-containing polypeptide.

[0145] Routes of Administration. Any efficacious route of administration may be used to therapeutically administer alpha-helix-containing polypeptides or antagonists thereof, including those compositions comprising nucleic acids. Parenteral administration includes injection, for example, via intra-articular, intravenous, intramuscular, intralesional, intraperitoneal or subcutaneous routes by bolus injection or by continuous infusion, and also includes localized administration, e.g., at a site of disease or injury. Other suitable means of administration include sustained release from implants; aerosol inhalation and/or insufflation; eyedrops; vaginal or rectal suppositories; buccal preparations; oral preparations, including pills, syrups, lozenges or chewing gum; and topical preparations such as lotions, gels, sprays, ointments or other suitable techniques. Alternatively, polypeptideaceous alpha-helix-containing polypeptides or antagonists may be administered by implanting cultured cells that express the polypeptide, for example, by implanting cells that express alpha-helix-containing polypeptides or antagonists. Cells may also be cultured ex vivo in the presence of polypeptides of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes. In another embodiment, the patient's own cells are induced to produce alpha-helix-containing polypeptides or antagonists by transfection in vivo or ex vivo with a DNA that encodes alpha-helix-containing polypeptides or antagonists. This DNA can be introduced into the patient's cells, for example, by injecting naked DNA or liposome-encapsulated DNA that encodes alpha-helix-containing polypeptides or antagonists, or by other means of transfection. Nucleic acids of the invention may also be administered to patients by other known methods for intro-

duction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA). When alpha-helix-containing polypeptides or antagonists are administered in combination with one or more other biologically active compounds, these may be administered by the same or by different routes, and may be administered simultaneously, separately or sequentially.

[0146] Oral Administration. When a therapeutically effective amount of polypeptide of the present invention is administered orally, polypeptide of the present invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% polypeptide of the present invention, and preferably from about 25 to 90% polypeptide of the present invention. When administered in liquid form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to 90% by weight of polypeptide of the present invention, and preferably from about 1 to 50% polypeptide of the present invention.

[0147] Intravenous Administration. When a therapeutically effective amount of polypeptide of the present invention is administered by intravenous, cutaneous or subcutaneous injection, polypeptide of the present invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable polypeptide solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to polypeptide of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of-the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the polypeptide of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

[0148] Bone and Tissue Administration. For compositions of the present invention which are useful for bone, cartilage, tendon or ligament disorders, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a polypeptide of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the polypeptide-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications. The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure polypeptides or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other ceramics Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability. Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the polypeptide compositions from disassociating from the matrix. A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethyl-cellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt %, preferably 1-10 wt % based on total formulation weight, which represents the amount necessary to prevent desorbtion of the polypeptide from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the polypeptide the opportunity to assist the osteogenic activity of the progenitor cells. In further compositions, polypeptides of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth

factors (TGF-alpha and TGF-beta), and insulin-like growth factor (IGF). The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with polypeptide's of the present invention. The dosage regimen of a polypeptide-containing pharmaceutical composition to be used in tissue regeneration will be determined by the attending physician considering various factors which modify the action of the polypeptides, e.g., amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (e.g., bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and with inclusion of other polypeptides in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

[0149] Veterinary Uses. In addition to human patients, alpha-helix-containing polypeptides and antagonists are useful in the treatment of disease conditions in non-human animals, such as pets (dogs, cats, birds, primates, etc.), domestic farm animals (horses cattle, sheep, pigs, birds, etc.), or any animal that suffers from a TNFα-mediated inflammatory or arthritic condition. In such instances, an appropriate dose may be determined according to the animal's body weight. For example, a dose of 0.2-1 mg/kg may be used. Alternatively, the dose is determined according to the animal's surface area, an exemplary dose ranging from 0.1-20 mg/m^2, or more preferably, from 5-12 mg/m^2. For small animals, such as dogs or cats, a suitable dose is 0.4 mg/kg. In a preferred embodiment, alpha-helix-containing polypeptides or antagonists (preferably constructed from genes derived from the same species as the patient), is administered by injection or other suitable route one or more times per week until the animal's condition is improved, or it may be administered indefinitely.

[0150] Manufacture of Medicaments. The present invention also relates to the use alpha-helix-containing polypeptides, fragments, and variants; nucleic acids encoding the alpha-helix-containing polypeptides, fragments, and variants; agonists or antagonists of the alpha-helix-containing polypeptides such as antibodies; alpha-helix-containing polypeptide binding partners; complexes formed from the alpha-helix-containing polypeptides, fragments, variants, and binding partners, etc, in the manufacture of a medicament for the prevention or therapeutic treatment of each medical disorder disclosed herein.

Use of Alpha-Helix-Containing polypeptides and Antagonists Thereof as Adjuvants

[0151] An effective vaccine must induce an appropriate immune response to the correct antigen or antigens. The immune system uses many mechanisms for attacking pathogens, but not all of these are activated after immunization. Protective immunity induced by vaccination is dependent on the capacity of the vaccine to elicit the appropriate immune response to resist, control, or eliminate the pathogen. Depending on the pathogen, this may require a humoral immune response, which involves antibodies and other factors such as complement, and/or a cell-mediated immune response, which is mediated by cells such as cytotoxic T cells. The type of immune response that is produced is determined by the nature of the T cells that develop after immunization. For example, many bacterial, protozoal, and intracellular parasitic and viral infections appear to require a strong cell-mediated immune response for protection, while other pathogens such as helminths primarily respond to a humoral response. The current paradigm of the role of T cells in the particular immune response is that $CD4^+$ T cells can be separated into subsets on the basis of the repertoire of cytokines produced and that the distinct cytokine profile observed in these cells determines their function. This T cell model includes two major subsets: Th1 cells that produce IL-2 and interferon gamma (IFN-gamma) and mediate cellular immune responses, and Th2 cells that produce IL-4, IL-5, and IL-10 and augment humoral immune responses (Mosmann et al., 1986, J Immunol 126: 2348).

[0152] Many vaccine compositions employ adjuvants, that is, substances which enhance the immune response when administered together with an immunogen or antigen. Adjuvants are thought to function in one or more of several possible ways, including increasing the surface area of antigen; prolonging the retention of the antigen in the body thus allowing time for the lymphoid system to have access to the antigen; slowing the release of antigen; targeting antigen to macrophages; increasing antigen uptake; up-regulating antigen processing; stimulating cytokine release; stimulating B cell switching and maturation and/or eliminating immuno-suppressor cells; activating macrophages, dendritic cells, B cells and T cells; or otherwise eliciting non-specific activation of the cells of the immune system (see, for example, Warren et al., 1986, Annu Rev Immunol 4: 369). Many of the most effective adjuvants include bacteria or their products, e.g., microorganisms such as the attenuated strain of *Mycobacterium bovis, bacillus* Calmette-Guerin (BCG); microorganism components, e.g., alum-precipitated diphtheria toxoid, bacterial lipopolysaccharide and endotoxins. Despite their immunostimulating properties, many bacterial adjuvants have toxic or other negative effects, particularly in humans. For example, such a large population has been exposed to some of the bacterial adjuvants, like BCG, that there is a danger of eliciting a secondary response with future use as a vaccine adjuvant. Heat-killed bacteria, being non-native to mammalian hosts, also risk causing toxic effects in the host. Alternative adjuvants that stimulate or enhance the host's immune responses without inducing a toxic effect, and which are suitable for use in pharmaceutical compositions, such as vaccines, are particularly useful. Also, an essential role of adjuvants in vaccines is to modulate $CD4^+$ T cell subset differentiation. The ability of an adjuvant to induce and increase a specific type of effector T cell (Th1 or Th2) and thus a specific type of immune response (cell-mediated or humoral) is a key factor in the selection of particular adjuvants for vaccine use against a particular pathogen. The present invention provides the use of alpha-helix-containing polypeptides and agonists thereof as adjuvants in vaccines, in order to promote the production of Th1 or Th2 cells by the vaccine, and/or to increase or modify the immunogenicity or the tolerance-inducing activity of the vaccine, which is useful

for example when the vaccine is meant to increase tolerance toward an allergenic antigen (or allergen).

[0153] Antigens are substances which are capable, under appropriate conditions, of inducing a specific immune response and of reacting with the products of that response, such as specific antibodies or T cells, or both. A vaccine is a composition comprising antigenic moieties, usually consisting of inactivated infectious agents or of allergens, or some part of an infectious agent or allergen, that is injected into the body to produce active immunity, or in the case of allergens, to induce tolerance. Antigens that can be used in the present invention are compounds which, when introduced into a mammal, preferably a human, will result in the formation of antibodies and/or cell-mediated immunity. Representative of the antigens that can be used according to the present invention include, but are not limited to live or killed viruses and other microorganisms; natural, recombinant or synthetic products derived from viruses, bacteria, fungi, parasites and other infectious agents; antigens promoting autoimmune diseases, hormones, or tumor antigens which might be used in prophylactic or therapeutic vaccines; and allergens (see the Table below). The viral or microorganismal products can be components which the organism produced by enzymatic cleavage or can be components of the organism (proteins, polypeptides, polysaccharides, nucleic acids, lipids, etc.) that were produced by recombinant DNA techniques that are well-known to those of ordinary skill in the art. The antigen component of the vaccine may also comprise one or several antigenic molecules such as haptens, which are small antigenic determinants capable of eliciting an immune response only when coupled to a carrier.

Antigen Category	Some Specific Examples of Representative Antigens
Viruses	Rotavirus; foot and mouth disease; influenza, including influenza A and B; parainfluenza; Herpes species (Herpes simplex, Epstein-Barr virus, chicken pox, pseudorabies, cytomegalovirus); rabies; polio; hepatitis A; hepatitis B; hepatitis C; hepatitis E; measles; distemper; Venezuelan equine encephalomyelitis; feline leukemia virus; reovirus; respiratory syncytial virus; bovine respiratory syncytial virus; Lassa fever virus; polyoma tumor virus; parvovirus; canine parvovirus; papilloma virus; tick-borne encephalitis; rinderpest; human rhinovirus species; enterovirus species; Mengo virus; paramyxovirus; avian infectious bronchitis virus; HTLV 1; HIV-1; HIV-2; LCMV (lymphocytic choriomeningitis virus); adenovirus; togavirus (rubella, yellow fever, dengue fever); corona virus
Bacteria	*Bordetella pertussis*; *Brucella abortis*; *Escherichia coli*; *Salmonella* species including *Salmonella typhi*; *streptococci*; *Vibrio* species (*V. cholera*, V. *parahaemolyticus*); *Shigella* species; *Pseudomonas* species; *Brucella* species; *Mycobacteria* species (tuberculosis, avium, BCG, leprosy); *pneumococci*; *staphylococci*; *Enterobacter* species; *Rochalimaia henselae*; *Pasterurella* species (*P. haemolytica*, *P. multocida*); *Chlamydia* species (*C. trachomatis*, *C. psittaci*, *Lymphogranuloma venereum*); Syphilis (*Treponema pallidum*); *Haemophilus* species; *Mycoplasma* species; Lyme disease (*Borrelia burgdorferi*); Legionnaires' disease; Botulism (*Colstridium botulinum*); *Corynebacterium diphtheriae*; *Yersinia entercolitica*
Ricketsial Infections	Rocky mountain spotted fever; thyphus; *Ehrlichia* species
Parasites and Protozoa	Malaria (*Plasmodium falciparum*, *P. vivax*, *P. malariae*); schistosomes; trypanosomes; *Leishmania* species; filarial nematodes; trichomoniasis; sarcosporidiasis; *Taenia* species (*T. saginata*, *T. solium*); *Toxoplasma gondii*; trichinelosis (*Trichinella spiralis*); coccidiosis (*Eimeria* species)
Fungi	*Cryptococcus neoformans*; *Candida albicans*; *Apergillus fumigatus*; coccidioidomycosis
Recombinant Proteins	Herpes simplex; Epstein-Barr virus; hepatitis B; pseudorabies; flavivirus (dengue, yellow fever);*Neisseria gonorrhoeae*; malaria: circumsporozoite protein, merozoite protein; trypanosome surface antigen protein; pertussis; alphaviruses; adenovirus
Proteins	Diphtheria toxoid; tetanus toxoid; meningococcal outer membrane protein (OMP); streptococcal M protein; hepatitis B; influenza hemagglutinin; cancer antigen; tumor antigens; toxins; exotoxins; neurotoxins; cytokines and cytokine receptors; monokines and monokine receptors
Synthetic Peptides	Malaria; influenza; foot and mouth disease virus; hepatitis B; hepatitis C
Polysaccharides	Pneumococcal polysaccharide; Haemophilis influenza polyribosyl-ribitolphosphate (PRP); *Neisseria meningitides*; *Pseudomonas aeruginosa*; *Klebsiella pneumoniae*
Oligosaccharide	Pneumococcal
Allergens	Plant pollens; animal dander; dust mites, *Blatella* species antigens (Bla g 1, 2, or 5), *Periplaneta* species antigens (Per a 1)

[0154] Adjuvants are compounds that, when used in combination with specific vaccine antigens, augment or otherwise alter or modify the resultant immune responses. Modification of the immune response means augmenting, intensifying, or broadening the specificity of either or both antibody and cellular immune responses. Modification of the immune response can also mean decreasing or suppressing certain antigen-specific immune responses, for example, in the induction of tolerance toward an allergen. Modification of the immune response by the adjuvant may increase the overall titer of antibodies specific for the vaccine antigen and/or induce cellular immune responses specific for the vaccine antigen, so that effective vaccination can be made

using lower amounts of antigen. Methods for detecting modification of the immune response by the adjuvant include several well-known assays such as ELISA (enzyme-linked immunosorbent assay), which measures the titer of antigen-specific antibodies, and the ELISPOT (enzyme-linked immunospot) assay, which allows ex vivo quantification of antigen-reactive T cells and of cells producing antigen-specific antibodies (see, for example, Zigterman et al., 1988, *J Immunol Methods* 106: 101-107; U.S. Pat. No. 6,149,922; and U.S. Pat. No. 6,153,182). Variations of ELISA in which biotin/avidin interactions are used to create antibody-antigen-antibody 'bridges' or 'sandwiches' are also well known in the art (see, for example, U.S. Pat. No. 6,149,922). In order to measure the effect of an adjuvant preparation on the production of functional, neutralizing antibodies, influenza virus hemagglutinin (HA) can be used as an antigen, animals are immunized with HA with differing amounts of adjuvant, and the ability of the resulting serum antibodies to inhibit the hemagglutinin-dependent agglutination of red blood cells can be determined using a hemagglutination inhibition (HAI) assay, essentially as described by the CDC Manual (U.S. Department of Health and Human Services/Public Health Service/Centers for Disease Control, 1982, *Concepts and Procedures for Laboratory Based Influenza Surveillance*) and U.S. Pat. No. 6,149,922. These assays allow the effects of supplementing a vaccine with alpha-helix-containing polypeptides or antagonists to be investigated by determining antibody titers and the kinetics of antibody responses. For example, dose-titration studies of a vaccine can be done to identify doses that induce measurable antibody responses after a single immunization. Antibody responses are followed for 30, 60, or 90 or more days and dose levels that are optimally and suboptimally immunogenic can be identified. Also, vaccine formulations containing these dose levels and supplemented with increasing amounts of adjuvant (alpha-helix-containing polypeptide or antagonist) can be evaluated and active doses of adjuvant identified. The kinetics and duration of antibody responses can evaluated by extension of the observation and antibody testing period to 6 months or more (see, for example, U.S. Pat. No. 6,149,922). Modulation of the immune response by adjuvant can also be assessed by measuring the antigen-dependent proliferation of T cells from immunized mice in a ^{3}H-thymidine uptake assay (see, for example, U.S. Pat. No. 6,051,227 and U.S. Pat. No. 6,153,182). Other T cell responses to immunization with varying amounts of adjuvant can be measured by determining the profile of cytokines secreted by T cells isolated from immunized animals, which may indicate whether Th1 or Th2 effector T cells are preferentially produced, or by assaying for functional cytotoxic T cells (see, for example, U.S. Pat. No. 6,149,922).

[0155] When used as an adjuvant in a vaccine composition, alpha-helix-containing polypeptides or antagonists are desirably admixed as part of the vaccine composition itself. One of skill in the art of vaccine composition can readily determine suitable amounts of alpha-helix-containing polypeptides or antagonists to adjuvant particular vaccines. Such amounts will depend upon the purpose for which the vaccine is designed, the nature of the antigen, and the dosage amounts of the antigen, as well as the species and physical and medical conditions of the vaccinate. As one example, an effective adjuvanting amount of a alpha-helix-containing polypeptide or antagonist is desirably between about 0.01 micrograms to about 10 mg (preferably about 0.1 microgram to about 1 mg, and more preferably about 1 microgram to about 0.1 mg) of alpha-helix-containing polypeptide polypeptide or antagonist per about 25 micrograms of antigen. When administered as part of a vaccine composition, alpha-helix-containing polypeptides or antagonists are administered by the same route as the vaccinal antigen. Any route of administration can be employed for the administration of this vaccine, e.g., subcutaneous, intraperitoneal, oral, intramuscular, intranasal and the like. The adjuvants may be given orally in alkaline solutions in vaccines appropriate for raising mucosal antibodies against antigens which give rise to intestinal diseases, as alkaline solutions such as those containing bicarbonates protect antigens and adjuvants from destruction in the upper GI tract. Alternatively, the adjuvanting effect of alpha-helix-containing polypeptides or antagonists can be employed by administering alpha-helix-containing polypeptides or antagonists separately from the vaccine composition, and preferably in the presence of a suitable carrier, such as saline and optionally conventional pharmaceutical agents enabling gradual release of the alpha-helix-containing polypeptide or antagonist. The amount of the alpha-helix-containing polypeptides or antagonists used in this mode of vaccination is similar to the ranges identified above when alpha-helix-containing polypeptides or antagonists are part of the vaccine composition. The alpha-helix-containing polypeptides or antagonists can be administered contemporaneously with the vaccine composition, either simultaneously therewith, or before the vaccine antigen administration. If the alpha-helix-containing polypeptide or antagonist is administered before the vaccine composition, it is desirable to administer it about one or more days before the vaccine. When alpha-helix-containing polypeptides or antagonists are administered as a separate component from the vaccine, they are desirably administered by the same route as the vaccinal antigen, e.g., subcutaneous route, or any other route as selected by a physician.

[0156] In addition to the administration of alpha-helix-containing polypeptides or antagonists as an adjuvant, nucleic acid sequences encoding alpha-helix-containing polypeptides or antagonists or a fragment thereof can also be used as an adjuvant. The nucleic acid sequences, preferably in the form of DNA, can be delivered to a vaccinate for in vivo expression of the alpha-helix-containing polypeptide or antagonist. Naked DNA can also be used to express the alpha-helix-containing polypeptides or antagonists in a patient (see, for example, Cohen, 1993, *Science* 259: 1691-1692; Fynan et al., 1993, *Proc Natl Acad Sci* 90: 11478-11482; and Wolff et al., 1991, *Biotechniques* 11: 474-485). For example, alpha-helix-containing polypeptide DNA can be incorporated into a microorganism itself, if it as a whole pathogen is to be employed as the vaccinal antigen. Alternatively, alpha-helix-containing polypeptide DNA can be administered as part of the vaccine composition or separately, but contemporaneously with the vaccine antigen, e.g., by injection. Still other modes of delivering alpha-helix-containing polypeptide or antagonist to the vaccinate in the form of DNA are known to those of skill in the art and can be employed rather than administration of the alpha-helix-containing polypeptide or antagonist, as desired. For example, alpha-helix-containing polypeptide DNA can be administered as part of a vector or as a cassette containing the alpha-helix-containing polypeptide DNA sequences operatively linked to a promoter sequence. When alpha-helix-containing polypeptide nucleic acid sequences are

used as an adjuvant, these sequences can be operably linked to DNA sequences which encode the antigen. Hence, the vector or cassette, as described above, encoding the alpha-helix-containing polypeptide DNA sequences can additionally include sequences encoding the antigen. Each of these sequences can be operatively linked to the promoter sequence of the vector or cassette. Alternatively, naked DNA encoding the antigen can be in a separate plasmid. Where present in one or two plasmids, the naked DNA encoding the antigen and/or alpha-helix-containing polypeptide or antagonist, upon introduction into the host cells, permits the infection of the vaccinate's cells and expression of both antigen and alpha-helix-containing polypeptide or antagonist in vivo. When alpha-helix-containing polypeptide nucleic acid sequences are employed as the adjuvant, the amounts of DNA to be delivered and the routes of delivery may parallel the alpha-helix-containing polypeptide or antagonist amounts and delivery described above, and can also be determined readily by one of skill in the art. Similarly the amounts of the antigen-encoding DNA can be selected by one of skill in the art.

EXAMPLES

[0157] The following examples are intended to illustrate particular embodiments and not to limit the scope of the invention.

Example 1

Identification of New Alpha-Helix-Containing Polypeptides

[0158] A data set was received from Celera Genomics (Rockville, Md.) containing a listing of amino acid sequences predicted to be encoded by the human genome. This data set was searched with a BLAST algorithm to identify cytokine family polypeptides. SEQ ID NOs 1 through 10 are a set of amino acid sequences that were identified as having alpha helices arranged in structures similar to known 4AHB cytokines by using the GeneFold programs described above on the data received from Celera Genomics, in combination with other analytical methods. Additional analysis has identified polypeptides comprising the amino acid sequences of SEQ ID NOs 12, 14, 16, 18, and 20; which are encoded by nucleotides 91 through 978 of SEQ ID NO:11, nucleotides 172 through 1155 of SEQ ID NO:13 (nucleotides 1156 through 1158 of SEQ ID NO:13 are a stop codon), and by open reading frames starting with the first nucleotide of SEQ ID NOs 15, 17, and 19, respectively. SEQ ID NO:12 is a partial human polypeptide corresponding to the full-length murine polypeptide of SEQ ID NO:14; these polypeptides are also referred to as human and murine IMX130124 polypeptides, respectively. SEQ ID NO:16 and SEQ ID NO:18 are two closely related human partial polypeptides with SEQ ID NO:20 being a corresponding partial murine polypeptide; these polypeptides are also referred to as human IMX129990a, human IMX129990b, and murine IMX129990 polypeptides, respectively.

Example 2

Analysis of Expression of Alpha-Helix-Containing Polypeptides by Real-Time Quantitative PCR

[0159] RNA samples were obtained from a variety of tissue sources and from cells or tissues treated with a variety of compounds; these RNA samples included commercially available RNA (Ambion, Austin, Tex.; Clontech Laboratories, Palo Alto, Calif.; and Stratagene, La Jolla, Calif.). The RNA samples were DNase treated (part # 1906, Ambion, Austin, Tex.), and reverse transcribed into a population of cDNA molecules using TaqMan Reverse Transcription Reagents (part # N808-0234, Applied Biosystems, Foster City, Calif.) according to the manufacturer's instructions using random hexamers. Each population of cDNA molecules was placed into specific wells of a multi-well plate at either 5 ng or 20 ng per well and run in triplicate. Pooling was used when same tissue types and stimulation conditions were applied but collected from different donors. Negative control wells were included in each multi-well plate of samples.

[0160] Sets of probes and oligonucleotide primers complementary to mRNAs encoding human alpha-helix-containing polypeptides (SEQ ID NOs 12 and 16) were designed using Primer Express software (Applied Biosystems, Foster City, Calif.) and synthesized, and PCR conditions for these probe/primer sets were optimized to produce a steady and logarithmic increase in PCR product every thermal cycle between approximately cycle 20 and cycle 36. Oligonucleotide primer sets complementary to 18S RNA and to mRNAs encoding certain 'housekeeper' proteins—beta-actin, HPRT (hypoxanthine phosphoribosyltransferase), DHFR (dihydrofolate reductase), PKG (phosphoglycerate kinase), and GAPDH (glyceraldehyde-3-phosphate dehydrogenase)—were synthesized and PCR conditions were optimized for these primer sets also. Multiplex TAQMAN PCR reactions using primers for message encoding SEQ ID NO:12 polypeptide and beta-actin probe/primer sets, or primers for message encoding SEQ ID NO:14 polypeptide and beta-actin probe/primer sets, were set up in 25-microliter volumes with TAQMAN Universal PCR Master Mix (part # 4304437, Applied Biosystems, Foster City, Calif.) on an Applied Biosystems Prism 7700 Sequence Detection System. Threshold cycle values (C_T). were determined using Sequence Detector software version 1.7a (Applied Biosystems, Foster City, Calif.), and delta C_T was calculated and transformed to $2E(-dC_T)$, which is 2 to the minus delta C_T, for relative expression comparison of the human alpha-helix-containing polypeptides to beta-actin.

[0161] Expression of human alpha-helix-containing polypeptides relative to beta-actin expression was analyzed in a variety of adult and fetal RNA samples. This analysis indicated that human message for SEQ ID NO:12 polypeptide is detectable (although less abundant than beta-actin) in certain adult and fetal tissues, such as-small intestine, testis, prostate, thyroid, fetal stomach, fetal colon, and fetal brain, with the highest ratio of expression (0.00043101) in testis; a ratio of 0.00043101 indicates that the expression of SEQ ID NO:12 polypeptide in this sample is about 0.04% of that of beta-actin. There was also detectable relative expression of SEQ ID NO:12 polypeptide in human MG63 osteoblast-like cells at various stages of differentiation with or without treatments such as vitamin D; however the highest level of relative expression detected was 0.0008% of that of beta-actin.

[0162] With respect to relative expression of the SEQ ID NO:16 polypeptide, this polypeptide showed expression in most tissues tested at a level of 0.002-0.04% of beta actin, but with the highest level in testis 0.4% of the level of beta

actin expression, approximately ten-fold higher than in the next most abundant tissue source. Expression of SEQ ID NO:16 polypeptide was also detected in peripheral blood mononuclear cells treated with anti-CD3; in natural killer cells treated with IL-15; in unstimulated T84 cells; in liver cells treated with a combination of IL-1, IL-18, and TNF; and in SAEC treated with interferon gamma, the last instance producing the highest expression level for these experiments: 0.004% of housekeeper expression.

Example 3

Monoclonal Antibodies That Bind Polypeptides of the Invention

[0163] This example illustrates a method for preparing monoclonal antibodies that bind alpha-helix-containing polypeptides. Other conventional techniques may be used, such as those described in U.S. Pat. No. 4,411,993. Suitable immunogens that may be employed in generating such antibodies include, but are not limited to, purified alpha-helix-containing polypeptide, an immunogenic fragment thereof, and cells expressing high levels of alpha-helix-containing polypeptide or an immunogenic fragment thereof. DNA encoding a alpha-helix-containing polypeptide can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3: 165, 1995.

[0164] Rodents (BALB/c mice or Lewis rats, for example) are immunized with alpha-helix-containing 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, Mont.)), and injected in amounts ranging from 10-100 micrograms subcutaneously or intraperitoneally. DNA may be given intradermally (Raz et al., 1994, *Proc. Natl. Acad. Sci. USA* 91: 9519) or intamuscularly (Wang et al., 1993, *Proc. Natl. Acad. Sci USA* 90: 4156); saline has been found to be a suitable diluent for DNA-based antigens. Ten days to three weeks days later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

[0165] Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision to test for alpha-helix-containing polypeptide antibodies by dot-blot assay, ELISA (enzyme-linked immunosorbent assay), immunoprecipitation, or other suitable assays, such as FACS analysis of inhibition of binding of alpha-helix-containing polypeptide to a alpha-helix-containing polypeptide binding partner. Following detection of an appropriate antibody titer, positive animals are provided one last intravenous injection of alpha-helix-containing polypeptide in saline. Three to four days later, the animals are sacrificed, and spleen cells are harvested and fused to a murine myeloma cell line, e.g., NS1 or preferably P3X63Ag8.653 (ATCC CRL-1580). These cell fusions generate hybridoma cells, which are 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.

[0166] The hybridoma cells may be screened by ELISA for reactivity against purified alpha-helix-containing polypeptide by adaptations of the techniques disclosed in Engvall et al., (*Immunochem.* 8: 871, 1971) and in U.S. Pat. No. 4,703,004. A preferred screening technique is the antibody capture technique described in Beckmann et al., (*J. Immunol.* 144: 4212, 1990). Positive hybridoma cells can be injected intraperitoneally into syngeneic rodents to produce ascites containing high concentrations (for example, greater than 1 milligram per milliliter) of anti-cytokine polypeptide monoclonal antibodies. Alternatively, hybridoma cells can be grown in vitro in flasks or roller bottles by various techniques. Monoclonal antibodies can be purified by ammonium sulfate precipitation, followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to cytokine polypeptide.

Example 4

Antisense Inhibition of Alpha-Helix-Containing Nucleic Acid Expression

[0167] In accordance with the present invention, a series of oligonucleotides are designed to target different regions of the mRNA molecule encoding the alpha-helix-containing polypeptide, using nucleotide sequences encoding one or more amino acid sequences of the invention as the basis for the design of the oligonucleotides. The oligonucleotides are selected to be approximately 10, 12, 15, 18, or more preferably 20 nucleotide residues in length, and to have a predicted hybridization temperature that is at least 37 degrees C. Preferably, the oligonucleotides are selected so that some will hybridize toward the 5' region of the mRNA molecule, others will hybridize to the coding region, and still others will hybridize to the 3' region of the mRNA molecule.

[0168] The oligonucleotides may be oligodeoxynucleotides, with phosphorothioate backbones (internucleoside linkages) throughout, or may have a variety of different types of internucleoside linkages. Generally, methods for the preparation, purification, and use of a variety of chemically modified oligonucleotides are described in U.S. Pat. No. 5,948,680. As specific examples, the following types of nucleoside phosphoramidites may be used in oligonucleotide synthesis: deoxy and 2'-alkoxy amidites; 2'-fluoro amidites such as 2'-fluorodeoxyadenosine amidites, 2'-fluorodeoxyguanosine, 2'-fluorouridine, and 2'-fluorodeoxycytidine; 2'-O-(2-methoxyethyl)-modified amidites such as 2,2'-anhydro[1-(beta-D-arabino-furanosyl)-5-methyluridine], 2'-O-methoxyethyl-5-methyluridine, 2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine, 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyluridine, 3'-O-acetyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methyl-4-triazoleuridine, 2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine, N4-benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine, and N4-benzoyl-2'-O-methoxyethyl-5'-O-dimethoxytrityl-5-methylcytidine-3'-amidite; 2'-O-(aminooxyethyl) nucleoside amidites and 2'-O-(dimethylaminooxyethyl) nucleoside amidites such as 2'-(dimethylaminooxyethoxy) nucleoside amidites, 5'-O-tert-butyldiphenylsilyl-O^2-2'-anhydro-5-methyluridine, 5'-O-tert-butyl-diphenylsilyl-2'-O-(2-hydroxyethyl)-5-methyluridine, 2'-O-([2-phthalimidoxy)ethyl]-5'-t-butyldiphenyl-silyl-5-methyluridine, 5'-O-tert-butyldiphenylsilyl-2'-O-[(2-formadoximinooxy)ethyl]-5-methyluridine, 5'-O-tert-butyldiphenylsilyl-2'-O-[N,N-dimethylaminooxyethyl]-5-

methyluridine, 2'-O-(dimethylaminooxy-ethyl)-5-methyluridine, 5'-O-DMT-2'-O-(dimethylaminooxyethyl)-5-methyluridine, and 5'-O-DMT-2'-O-(2-N,N-dimethylaminooxyethyl)-5-methyluridine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite]; and 2'-(aminooxyethoxy) nucleoside amidites such as N2-isobutyryl-6-O-diphenylcarbamoyl-2'-O-(2-ethylacetyl)-5'-O-(4,4'-dimethoxytrityl)guanosine-3'-[(2-cyanoethyl)-N,N-diisopropylphosphoramidite].

[0169] Modified oligonucleosides may also be used in oligonucleotide synthesis, for example methylenemethylimino-linked oligonucleosides, also called MMI-linked oligonucleosides; methylene-dimethylhydrazo-linked oligonucleosides, also called MDH-linked oligonucleosides; methylene-carbonylamino-linked oligonucleosides, also called amide-3-linked oligonucleosides; and methylene-aminocarbonyl-linked oligonucleosides, also called amide-4-linked oligonucleosides, as well as mixed backbone compounds having, for instance, alternating MMI and P═O or P═S linkages, which are prepared as described in U.S. Pat. Nos. 5,378,825, 5,386,023, 5,489,677, 5,602,240 and 5,610,289. Formacetal- and thioformacetal-linked oligonucleosides may also be used and are prepared as described in U.S. Pat. Nos. 5,264,562 and 5,264,564; and ethylene oxide linked oligonucleosides may also be used and are prepared as described in U.S. Pat. No. 5,223,618. Peptide nucleic acids (PNAs) may be used as in the same manner as the oligonucleotides described above, and are prepared in accordance with any of the various procedures referred to in Peptide Nucleic Acids (PNA): Synthesis, Properties and Potential Applications, Bioorganic & Medicinal Chemistry, 1996,4, 5-23; and U.S. Pat. Nos. 5,539,082, 5,700,922, and 5,719,262.

[0170] Chimeric oligonucleotides, oligonucleosides, or mixed oligonucleotides/oligonucleosides of the invention can be of several different types. These include a first type wherein the "gap" segment of linked nucleosides is positioned between 5' and 3'"wing" segments of linked nucleosides and a second "open end" type wherein the "gap" segment is located at either the 3' or the 5' terminus of the oligomeric compound. Oligonucleotides of the first type are also known in the art as "gapmers" or gapped oligonucleotides. Oligonucleotides of the second type are also known in the art as "hemimers" or "wingmers". Some examples of different types of chimeric oligonucleotides are: [2'-O-Me]-[2'-deoxy]-[2'-O-Me] chimeric phosphorothioate oligonucleotides, [2'-O-(2-methoxyethyl)]-[2'-deoxy]-[2'-O-(methoxyethyl)] chimeric phosphorothioate oligonucleotides, and [2'-O-(2-methoxyethyl)phosphodiester]-[2'-deoxy phosphoro-thioate]-[2'-O-(2-methoxyethyl)phosphodiester] chimeric oligonucleotides, all of which may be prepared according to U.S. Pat. No. 5,948,680. In one preferred embodiment, chimeric oligonucleotides ("gapmers") 18 nucleotides in length are utilized, composed of a central "gap" region consisting of ten 2'-deoxynucleotides, which is flanked on both sides (5' and 3' directions) by four-nucleotide "wings". The wings are composed of 2'-methoxyethyl (2'-MOE) nucleotides. The internucleoside (backbone) linkages are phosphorothioate (P═S) throughout the oligonucleotide. Cytidine residues in the 2'-MOE wings are 5-methylcytidines. Other chimeric oligonucleotides, chimeric oligonucleosides, and mixed chimeric oligonucleotides/oligonucleosides are synthesized according to U.S. Pat. No. 5,623,065.

[0171] Oligonucleotides are preferably synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer capable of assembling-96 sequences simultaneously in a standard 96 well format. The concentration of oligonucleotide in each well is assessed by dilution of samples and UV absorption spectroscopy. The full-length integrity of the individual products is evaluated by capillary electrophoresis, and base and backbone composition is confirmed by mass analysis of the compounds utilizing electrospray-mass spectroscopy.

[0172] The effect of antisense compounds on target nucleic acid expression can be tested in any of a variety of cell types provided that the target nucleic acid is present at measurable levels. This can be routinely determined using, for example, PCR or Northern blot analysis. Cells are routinely maintained for up to 10 passages as recommended by the supplier. When cells reached 80% to 90% confluency, they are treated with oligonucleotide. For cells grown in 96-well plates, wells are washed once with 200 microliters OPTI-MEM-1 reduced-serum medium (Gibco BRL) and then treated with 130 microliters of OPTI-MEM-1 containing 3.75 g/mL LIPOFECTIN (Gibco BRL) and the desired oligonucleotide at a final concentration of 150 nM. After 4 hours of treatment, the medium is replaced with fresh medium. Cells are harvested 16 hours after oligonucleotide treatment. Preferably, the effect of several different oligonucteotides should be tested simultaneously, where the oligonucleotides hybridize to different portions of the target nucleic acid molecules, in order to identify the oligonucleotides producing the greatest degree of inhibition of expression of the target nucleic acid.

[0173] Antisense modulation of nucleic acid expression can be assayed in a variety of ways known in the art. For example, mRNA levels can be quantitated by, e.g., Northern blot analysis, competitive polymerase chain reaction (PCR), or real-time PCR (RT-PCR). Real-time quantitative PCR is presently preferred. RNA analysis can be performed on total cellular RNA or poly(A)+ mRNA. Methods of RNA isolation and Northern blot analysis are taught in, for example, Ausubel, F. M. et al., Current Protocols in Molecular Biology, Volume 1, pp. 4.1.1-4.2.9 and 4.5.1-4.5.3, John Wiley & Sons, Inc., 1996. Real-time quantitative (PCR) can be conveniently accomplished using the commercially available ABI PRISM 7700 Sequence Detection System, available from PE-Applied Biosystems, Foster City, Calif. and used according to manufacturer's instructions. This fluorescence detection system allows high-throughput quantitation of PCR products. As opposed to standard PCR, in which amplification products are quantitated after the PCR is completed, products in real-time quantitative PCR are quantitated as they accumulate. This is accomplished by including in the PCR reaction an oligonucleotide probe that anneals specifically between the forward and reverse PCR primers, and contains two fluorescent dyes. A reporter dye (e.g., JOE or FAM, obtained from either Operon Technologies Inc., Alameda, Calif. or PE-Applied Biosystems, Foster City, Calif.) is attached to the 5' end of the probe and a quencher dye (e.g., TAMRA, obtained from either Operon Technologies Inc., Alameda, Calif. or PE-Applied Biosystems, Foster City, Calif.) is attached to the 3' end of the probe. When the probe and dyes are intact, reporter dye emission is quenched by the proximity of the 3' quencher dye. During amplification, annealing of the probe to the target sequence creates a substrate that can be cleaved by the

5'-exonuclease activity of Taq polymerase. During the extension phase of the PCR amplification cycle, cleavage of the probe by Taq polymerase releases the reporter dye from the remainder of the probe (and hence from the quencher moiety) and a sequence-specific fluorescent signal is generated. With each cycle, additional reporter dye molecules are cleaved from their respective probes, and the fluorescence intensity is monitored at regular (six-second) intervals by laser optics built into the ABI PRISM 7700 Sequence Detection System. In each assay, a series of parallel reactions containing serial dilutions of mRNA from untreated control samples generates a standard curve that is used to quantitate the percent inhibition after antisense oligonucleotide treatment of test samples. Other methods of quantitative PCR analysis are also known in the art. Alpha-helix-containing protein levels can be quantitated in a variety of ways well known in the art, such as immunoprecipitation, Western blot analysis (immunoblotting), ELISA, or fluorescence-activated cell sorting (FACS). Antibodies directed to cytokine can be prepared via conventional antibody generation methods such as those described herein. Immunoprecipitation methods, Western blot (immunoblot) analysis, and enzyme-linked immunosorbent assays (ELISA) are standard in the art (see, for example, Ausubel, F. M. et al., Current Protocols in Molecular Biology, Volume 2, pp. 10.16.1-10.16.11, 10.8.1-10.8.21, and 11.2.1-11.2.22, John Wiley & Sons, Inc., 1991).

[0174] All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 20

<210> SEQ ID NO 1
<211> LENGTH: 252
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 1

```
Met Ser Ser Lys Gln Val Arg Phe Thr Ser Gly Pro Gln Lys Arg Ser
1               5                   10                  15

Leu Gly Leu Asn Ile Asn Leu Gly Val Tyr Ser Arg Leu Met Phe Asn
            20                  25                  30

His Gly Val Ala Cys Gly His Glu Ile Gly Gly Ala Lys Ile Arg Ala
        35                  40                  45

Gln Asp Phe Thr Leu Val Ile Pro Thr His Arg Gly Cys Ile Glu Glu
    50                  55                  60

Glu Glu Gln Val Ser Lys Thr Lys Glu Ser Lys Lys Leu Ser Pro Glu
65                  70                  75                  80

Lys Leu Glu Glu Ser Ser Val Ser Leu Ser Lys Ser Glu Lys Leu Trp
                85                  90                  95

Phe His Phe Ser Pro Ile Leu Ala Ile Lys Thr Glu Ala Cys Phe Asn
            100                 105                 110

Leu Lys Val Ser Val Thr Trp Asn Lys Gly Ile Pro Val Val Thr Pro
        115                 120                 125

Gly Asp Cys Thr Lys Ala Met Tyr Ala Val Pro Gln Ile Thr Gln Ser
    130                 135                 140

Ser Gly Leu Trp Leu Gly Tyr Ser Leu Lys Glu Ala Ile Glu Lys Arg
145                 150                 155                 160

Ser Pro Gln Leu Leu Val Phe Phe Arg Glu Ser Ala Ile Tyr Glu Asn
                165                 170                 175

Tyr Met Gln Asn Ser Glu Tyr Phe Arg Arg Leu His Ser Phe His Lys
            180                 185                 190

Leu Leu Arg Ala Phe Cys Asp Pro Arg Leu Gln Gly Ser Pro Gly Val
        195                 200                 205
```

-continued

```
Asn Ser Ile Gln Pro Glu Thr Pro Ser Asn Leu Leu Thr Val Ala Ala
    210                 215                 220

Thr Val Glu Phe Val Met Gln Gln Glu Ser Ala Gly Ala Lys Pro Trp
225                 230                 235                 240

Arg Lys Lys Asn Leu Leu Ile Leu Met Glu Asp Leu
                245                 250


<210> SEQ ID NO 2
<211> LENGTH: 195
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

Met His Lys Ala Thr Asp Val Met Arg Leu Asn Ser Leu Ala Leu Phe
1               5                   10                  15

Arg Met Trp Leu Arg Asn Asp Thr Gly His Thr Gly Val Glu Ser Tyr
            20                  25                  30

Gly Leu Phe Ser Met Glu Glu Ser Leu Ala Asn Thr Arg Arg Leu Phe
        35                  40                  45

Gly Ser Ser Ile Leu Val Lys Ser Leu Ser Ser His Tyr Leu Val Thr
    50                  55                  60

Lys Leu Thr Asn Gln Lys Leu Gln Gly Ser Tyr Thr Ala Lys Asn Thr
65                  70                  75                  80

Tyr Gly Ile Glu Leu Val Gln Val Arg Leu Glu Ser Tyr Lys Val Ile
                85                  90                  95

Met Gln Gln Pro Ser Pro Leu Ser Gly Gln Leu Leu Ile Val Ser Ser
            100                 105                 110

Gly Leu Ala Pro Met Lys Thr Ser Lys Ala Ser Gln Arg Tyr Arg Gly
        115                 120                 125

Ile Arg Arg Asn Ala Ser Gln Cys Tyr Leu Tyr Gln Glu Ser Leu Leu
    130                 135                 140

Leu Ser Asn Leu Asp Asp Ser Phe Ser Ala Asp Glu Thr Gly Asp Ser
145                 150                 155                 160

Asn Asp Pro Glu Gln Ile Phe Gln Asn Ile Gln Phe Gln Lys Asp Leu
                165                 170                 175

Met Ala Asn Ile Arg Cys Arg Pro Trp Thr Met Gly Gln Lys Leu Arg
            180                 185                 190

Ala Leu Arg
        195


<210> SEQ ID NO 3
<211> LENGTH: 253
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

Ala Leu Ser Glu His Pro Ala Ile Leu Phe Ser Ser Val Pro Thr Arg
1               5                   10                  15

Val Thr Asp Ser Ser Leu Leu Cys Pro Val Thr Ile Ser His Gln Ser
            20                  25                  30

Ser Leu Tyr Ile Ala Pro Phe Pro Thr His Asp Asn Ser Ala Leu Ser
        35                  40                  45

Gln Leu Leu Gln Thr Trp Ile Leu Ser Pro Ser Leu Ser Val Val Phe
    50                  55                  60
```

-continued

```
Thr Tyr Met Ser Val Tyr Tyr Arg Lys Tyr Cys Ile Tyr Phe Leu Ser
65                  70                  75                  80

Val Asp Lys Arg Ser Ser Ile Thr Ser Ile Gln Tyr Ser Lys Lys Leu
                85                  90                  95

Gln Lys Leu Asn Asn Phe Thr Tyr Ser Ser Cys Thr Asn Leu Ala Asp
            100                 105                 110

Lys Ile Asp His His Arg Ser Gln Thr Pro Gln His Leu Gln Lys Asp
        115                 120                 125

Asp His Gly Val His Gln Asp Ser Val Cys Lys Lys Lys Cys Trp Thr
    130                 135                 140

Ser Tyr Glu Arg Leu Leu Ile Leu Gln Glu Gly Arg Leu Pro Pro Thr
145                 150                 155                 160

Lys Ser Asn Gly His Gly Leu Val Lys Glu Lys Gly Phe Arg Lys Ser
                165                 170                 175

Tyr Leu Lys Ile Glu Val Trp Leu Lys Val Leu Ser Arg Gln Gly Gly
            180                 185                 190

Gln Leu Pro Val Ser Pro Cys Phe Gly Glu Ala Asn Ile Gly Ile Tyr
        195                 200                 205

Ile Arg Arg Asp Leu Ser Glu Met Gln Ser Gly Val Arg Gly Leu Ser
    210                 215                 220

Ala Ala Leu Arg Ser Phe Ser Ile Ala Leu Ala Ala Glu Asn His Leu
225                 230                 235                 240

Thr Gln Gly Tyr Val Pro Ser Thr Ala Ala Tyr Ile Gln
                245                 250


<210> SEQ ID NO 4
<211> LENGTH: 199
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

Met Ile Ala Arg Cys Leu Leu Ala Val Arg Ser Leu Arg Arg Cys Leu
1               5                   10                  15

Lys Asn Leu Leu Gly Ala Phe Ile Phe Cys His Ala Tyr Leu Ala Asp
            20                  25                  30

Arg Asp Met Glu Cys Leu Ser Gln Tyr Pro Ser Leu Ser Gln Leu Lys
        35                  40                  45

Glu Leu His Leu Ile His Ile Leu Met Trp Thr Thr Asn Leu Glu Pro
    50                  55                  60

Leu Gly Ala Leu Leu Glu Lys Val Ala Ala Thr Leu Glu Thr Leu Thr
65                  70                  75                  80

Leu Lys Asp Cys Gln Ile Gln Asp Ser Gln Leu Arg Leu Leu Leu Pro
                85                  90                  95

Ala Leu Ser His Cys Ser Gln Leu Thr Thr Phe Tyr Phe His Gly Asn
            100                 105                 110

Glu Thr Ser Met Asn Ala Leu Lys Asp Leu Leu Cys His Thr Gly Gly
        115                 120                 125

Leu Ser Lys Leu Gly Leu Glu Leu Tyr Pro Ser Arg Leu Glu Ser Leu
    130                 135                 140

Asp Asn Arg Gly His Ala Asn Trp Glu Ile Leu Ala Pro Ile Arg Ala
145                 150                 155                 160

Glu Leu Met Cys Thr Val Arg Glu Val Arg Gln Pro Lys Arg Ile Phe
```

-continued

```
                165                 170                 175

Phe Gly Pro Val Pro Cys Pro Ser Cys Gly Ser Trp Pro Ser Glu Lys
            180                 185                 190

Val Asp Leu His Leu Cys Ser
        195


<210> SEQ ID NO 5
<211> LENGTH: 266
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 5

Met Thr Cys Ala Leu Val Glu Ser Gly Lys Ser Gly Gln His Val Gly
1               5                   10                  15

Thr Pro Thr Val Met Thr Val Pro Leu Gln Glu Leu Ala Val Leu Leu
            20                  25                  30

Gly Arg His Pro Ala Ala Asn Ala Val Leu Asp Asn Pro Ser Ile Asn
        35                  40                  45

Gln Ile Ile Lys Gln Leu Arg Ala Gly Phe Arg Ser Ala Ser Asp Ile
    50                  55                  60

Ala Pro Gly Val Tyr Pro Lys Thr Pro Pro Gly Leu His Tyr Ile Ser
65                  70                  75                  80

His Met Gln Ser Pro Gln Ala Phe Pro Leu Pro Ser Gly Ser Gly Leu
                85                  90                  95

Ile Ser Lys Val Met Thr Ile Lys Ile Ser Leu Thr Ala Leu Phe Leu
            100                 105                 110

Glu Lys Ser Met Lys Gly Asp Pro Phe Pro Leu Arg Val Asp Glu Leu
        115                 120                 125

Ser Pro Val Val Ser Ala Ala Ala Trp Arg Lys Glu Arg Asp Tyr Phe
    130                 135                 140

Phe Lys Pro His His Tyr Thr Asp Gln Ala Ile Gly Asp Thr Lys Thr
145                 150                 155                 160

Thr Leu Pro Ile Tyr Gln Ile Leu Pro Tyr His Ser Ala Ile His His
                165                 170                 175

Ser Leu Arg Gln His Val Tyr His Ser Met Ile Gln Thr Lys Lys Glu
            180                 185                 190

Pro Glu Leu Ser Ala Gln Glu Pro Val Asn Thr Ser Ala Ala Ala Glu
        195                 200                 205

Asp Ser Arg Glu Ala Ser Leu Gly Ala Trp Gln Ala Pro Pro Gly Pro
    210                 215                 220

Thr Ala Trp Pro Phe Arg Leu Met Gly Leu Glu Leu Leu Phe Lys Glu
225                 230                 235                 240

Cys Thr Pro Gly Thr Lys Gly Pro Cys Leu Ser Pro Leu Cys Ser Gly
                245                 250                 255

Ile Ile Ala Ala Phe Lys Asp Val Val Glu
            260                 265


<210> SEQ ID NO 6
<211> LENGTH: 145
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

Met Ala Glu Ile Phe Phe Pro Gly Asp Lys Glu Tyr Phe Lys Lys Gly
```

-continued

```
1               5                   10                  15

Asp Leu Cys Ser Ile Lys Glu Leu Thr Leu Phe Glu Thr His Cys Glu
            20                  25                  30

Lys Phe Met Pro Glu Glu Tyr Leu Val Gln Phe Gln Asn Leu Ser Gln
        35                  40                  45

Asn Leu Ala Tyr Lys His Ala Ser Ser Val Val Ile Phe Leu His Cys
    50                  55                  60

Phe Asp Asn Tyr Phe Val Met Phe Ile Arg Ala Lys Leu Asn Gly Ala
65                  70                  75                  80

Cys Asn Phe Cys Asp His Phe Ala Met Leu Gly Pro Ser Val Asn Ala
                85                  90                  95

Lys Phe Ala His Arg Gly Glu Leu Thr Asn Arg Arg Met Glu Asn Phe
            100                 105                 110

Leu Ile Ile Asp Asp Ser Ser Gln Pro Val Lys Pro Ser Ser Leu Glu
        115                 120                 125

Phe Phe Leu Leu Leu Glu Phe Leu Ser Leu Gln Lys Ile Asn Val Val
    130                 135                 140

Phe
145


<210> SEQ ID NO 7
<211> LENGTH: 383
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

Met Asp Asn Arg Asp Val Ala Gly Lys Ala Asn Arg Trp Phe Gly Val
1               5                   10                  15

Ser Pro Pro Lys Ser Gly Lys Met Asn Met Asn Ile Leu His Gln Glu
            20                  25                  30

Gln Leu Ile Ala Gln Lys Lys Gly Glu Ile Gly Ser Lys Met Gln Gln
        35                  40                  45

Lys Ala Lys Gln Asn Glu Val Ala Ser Pro Gln Pro Pro Tyr Pro Gly
    50                  55                  60

Glu Ile Thr Asn Ala His Asn Ser Ser Ser Val Ser Asn Lys Phe Ala
65                  70                  75                  80

Asn Asp Gly Ser Phe Leu Gln Gln Phe Leu Lys Leu Gln Met Ala Gln
                85                  90                  95

Thr Ser Thr Asp Ala Pro His Pro Gln Arg Gln Gly Ala Gln Gly Ser
            100                 105                 110

Arg Asp Ser Glu Ser Asp Arg Glu Ile Gly Pro Phe Val Ala Glu Ala
        115                 120                 125

Gly Pro Glu Leu Glu Lys Val Ile Thr Glu Asp Tyr Lys Asp Asn Leu
    130                 135                 140

Ala Phe Pro Phe Leu His Gly Ala Pro Gly Glu Pro Ala Ser Ala Ala
145                 150                 155                 160

Thr Val Lys Arg Lys Pro Lys Ser Arg Trp Arg Pro Glu Glu Asn Lys
                165                 170                 175

Val Glu Leu Ser Pro Ala Glu Leu Val Gln Arg Asp Val Asp Ala Ser
            180                 185                 190

Pro Ser Pro Leu Ser Val Gln Asp Leu Lys Gly Leu Gly Tyr Glu Asn
        195                 200                 205
```

-continued

```
Gly Lys Ser Val Gly Leu Ala Gly Leu Thr Glu Leu Ser Cys Ser Gln
    210                 215                 220

Lys Gln Gln Leu Lys Asp Gln Gln Glu Met His Gln Met Tyr Asp Met
225                 230                 235                 240

Ile Met Gln His Lys Trp Ala Met Gln Asp Met Gln Leu Leu Trp Glu
                245                 250                 255

Lys Ala Val Gln Gln His Gln His Gly Tyr His Ser Asp Glu Glu Val
            260                 265                 270

Ser Ser Glu Leu Arg Thr Trp Glu His Gln Leu Gln Arg Met Glu Met
        275                 280                 285

Asn Lys Thr Arg Glu Trp Ala Glu Gln Leu Thr Lys Met Ser Arg Gly
    290                 295                 300

Lys His Phe Ile Gly Asp Phe Leu Pro Pro Asp Lys Leu Glu Lys Phe
305                 310                 315                 320

Met Glu Thr Phe Lys Ala Leu Lys Glu Gly Arg Glu Pro Asp Tyr Ser
                325                 330                 335

Glu Tyr Lys Glu Phe Lys Leu Thr Glu Glu Asn Val Leu Met Lys Met
            340                 345                 350

Gly Trp Lys Glu Gly Glu Gly Leu Ser Glu Pro Gly His Gln Glu Pro
        355                 360                 365

Gly Gln Gln Gly His His Arg Ser Gly Trp Cys Arg Leu Arg His
    370                 375                 380


<210> SEQ ID NO 8
<211> LENGTH: 256
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

Ala Leu Ser Gln Leu Gln Cys Gly Leu Leu Gly Ser Ala Glu Gln Ser
1               5                   10                  15

Phe Leu Gln Leu Glu Gln Glu Asn His Ser Leu Lys Arg Gln Asn Gln
            20                  25                  30

Glu Leu Arg Glu Gln Leu Gly Ala Leu Leu Gly Pro Gly Gln Gln Phe
        35                  40                  45

Leu Pro Leu Cys Pro Glu His Ser Ser Cys Thr Ala Leu Ala Trp Pro
    50                  55                  60

Pro Asp Pro Ala Gly Thr Gln Pro Leu Gly Asn Arg Ala Pro Leu Gln
65                  70                  75                  80

Leu Leu Arg Arg Glu Leu Cys Gln Gly Gln Glu Ala Phe Val Gln Gln
                85                  90                  95

Ser Gln Asn Glu Leu Gln Gln Ile Arg Leu Cys Phe Glu Arg Lys Lys
            100                 105                 110

Met Val Ile Thr Glu Val Trp Asp Asn Val Ala Glu Met His Met Ala
        115                 120                 125

Leu Asn Asn Gln Ala Thr Gly Leu Leu Asn Leu Lys Lys Asp Ile Arg
    130                 135                 140

Gly Val Leu Asp Gln Met Glu Asp Ile Gln Leu Glu Ile Leu Arg Glu
145                 150                 155                 160

Arg Ala Gln Cys Arg Thr Arg Ala Arg Lys Glu Lys Gln Met Ala Ser
                165                 170                 175

Met Ser Lys Gly Arg Pro Lys Leu Gly Ser Ser Lys Gly Leu Ala Gly
            180                 185                 190
```

-continued

```
Gln Leu Trp Leu Leu Thr Leu Arg Leu Leu Leu Gly Ala Leu Leu Val
        195                 200                 205

Trp Thr Ala Ala Tyr Val Tyr Val Val Asn Pro Thr Pro Phe Glu Gly
    210                 215                 220

Leu Val Pro Pro Leu Leu Ser Arg Ala Thr Val Trp Lys Leu Arg Ala
225                 230                 235                 240

Leu Leu Asp Pro Phe Leu Arg Leu Lys Val Asp Gly Phe Leu Pro Phe
                245                 250                 255


<210> SEQ ID NO 9
<211> LENGTH: 251
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

Ala Leu Ser Gln Leu Gln Cys Gly Pro Leu Gly Ser Ala Glu Gln Ser
1               5                   10                  15

Phe Leu Glu Gln Glu Asn His Ser Leu Lys Arg Gln Asn Gln Asp Leu
            20                  25                  30

Arg Glu Gln Leu Gly Ala Leu Leu Gly Pro Gly Gln Gln Phe Leu Pro
        35                  40                  45

Leu Cys Pro Glu His Ser Ser Cys Thr Ala Leu Ala Trp Pro Pro Asp
    50                  55                  60

Pro Ala Gly Thr Gln Pro Leu Gly Asn Arg Ala Pro Leu Gln Leu Leu
65                  70                  75                  80

Arg Arg Glu Leu Ser Gln Gly Gln Glu Ala Phe Val Gln Gln Ser Leu
                85                  90                  95

Asn Glu Leu Gln Gln Ile Arg Leu Cys Phe Glu Arg Lys Lys Met Val
            100                 105                 110

Ile Thr Glu Ala Trp Asp Asn Val Ala Glu Met His Val Ala Leu Asn
        115                 120                 125

Asn Gln Ala Thr Gly Leu Leu Asn Leu Lys Lys Asp Ile Trp Gly Val
    130                 135                 140

Leu Asp Gln Met Glu Asp Ile Gln Leu Glu Ile Leu Arg Glu Arg Ala
145                 150                 155                 160

Gln Cys His Thr Gln Ala Lys Lys Glu Pro Gln Met Ala Ser Met Ala
                165                 170                 175

Lys Gly Arg Pro Lys Leu Glu Ser Ser Lys Gly Leu Ala Gly Gln Leu
            180                 185                 190

Trp Leu Leu Ile Leu Arg Leu Leu Leu Gly Thr Leu Leu Val Ala Tyr
        195                 200                 205

Val Tyr Met Val Asn Pro Arg Pro Phe Glu Gly Leu Val Pro Pro Leu
    210                 215                 220

Leu Ser Arg Ala Thr Ile Trp Lys Leu Arg Ala Leu Leu Asp Pro Phe
225                 230                 235                 240

Leu Arg Leu Glu Val Asp Gly Phe Leu Ser Phe
                245                 250


<210> SEQ ID NO 10
<211> LENGTH: 249
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10
```

-continued

```
Met Glu Val Gly Ala Gly Gly Gln Cys Ser His Asp Asn Asp Leu Leu
1               5                   10                  15

Ala Val Gln Lys Ala Gly Ser Ser Gly Phe Gln Pro Val Pro Pro Pro
            20                  25                  30

Gln Pro Arg Pro Val Leu His Asp Ile Pro Glu Pro Gly Pro Glu Ala
        35                  40                  45

Asn Pro His Ala His Pro Asn Pro Gly His Arg Leu Ala Gly Arg Leu
    50                  55                  60

Ala Phe Val Ser Val Tyr Asp Pro Gly Gly Ala Leu Ala Leu Pro Lys
65                  70                  75                  80

Ala Ala Leu Ser Gln Leu Gln Cys Gly Pro Leu Gly Ser Ala Glu Gln
                85                  90                  95

Ser Phe Leu Glu Gln Glu Asn His Ser Leu Lys Arg Gln Asn Gln Asp
            100                 105                 110

Leu Arg Glu Gln Leu Gly Ala Leu Leu Gly Pro Gly Gln Gln Phe Leu
        115                 120                 125

Pro Cys Val Pro Asn Thr Gln Ala Ala Leu Leu Trp Pro Gly Arg Asp
    130                 135                 140

Pro Thr Phe Ser Gln Ala Trp Asp Asn Val Ala Glu Met His Val Ala
145                 150                 155                 160

Leu Asn Asn Gln Ala Thr Gly Leu Leu Asn Leu Lys Lys Asp Ile Trp
                165                 170                 175

Gly Val Leu Asp Gln Met Glu Asp Ile Gln Leu Glu Ile Leu Arg Leu
            180                 185                 190

Leu Ile Leu Arg Leu Leu Leu Gly Thr Leu Leu Val Ala Tyr Val Tyr
        195                 200                 205

Met Val Asn Pro Arg Pro Phe Glu Gly Leu Val Pro Pro Leu Leu Ser
    210                 215                 220

Arg Ala Thr Ile Trp Lys Leu Arg Ala Leu Leu Asp Pro Phe Leu Arg
225                 230                 235                 240

Leu Glu Val Asp Gly Phe Leu Ser Phe
                245
```

<210> SEQ ID NO 11
<211> LENGTH: 979
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

```
cttccattca gaaacctcgg aacagacctt tgcctgccaa cttcctagct ctctggccag     60

cttctgattg ttagcagtgg gttagctccc atgaaaacct cgaaggcatc ccagcgctat    120

agaggcatcc ggagaaatgc cagccagtgc tacctctacc aggaatctct gctgctcagc    180

aacttggatg acagctttag tgctgatgaa acaggggaca gcaatgatcc ggaacaaatc    240

ttccagaata tacagttcca gaaagatctc atggcaaaca ttcgctgcag accctggact    300

atgggacaga agctgagggc attgagacaa gcgaagaaca ttgtgctgaa gtttgaaggg    360

aggctgacca ggacccgagg ctaccaagca gcaggtgcag agctctggcg gaaatttgct    420

cgtctcgcct gtaactttgt ggtcatcttc attccctggg aaatgaggat aaagaaaatc    480

gagagtcatt ttggatctgg cgtcgcctcc tatttcatat tcttgagatg gttatttgga    540

attaatattg tgctcaccat tatgacaggt gcttttattg tcattccaga gctgattgca    600
```

-continued

ggccagccct ttggaagcac agccaggaag accatcccca aggagcaggt ttcgtctgcc 660

caagacctgg acaccgtctg gtctctgggg ggctacctcc agtactctgt cctcttctac 720

ggatattacg gcagggagag gaagatcggg agagctggct accggctgcc cttggcgtat 780

ttcctagtgg ggatggcagt gtttgcttac agcttcatca ttcttttaaa aaagatggct 840

aagaactccc gcacgagtct tgccagtgct tccaatgaaa actatacctt ctgctggcgg 900

gtgttctgtg cctgggatta cctcattgga aacccagagg ctgcagagag caaaacagct 960

gccatagtga acagcatca 979

<210> SEQ ID NO 12
<211> LENGTH: 296
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

Met Lys Thr Ser Lys Ala Ser Gln Arg Tyr Arg Gly Ile Arg Arg Asn
1 5 10 15

Ala Ser Gln Cys Tyr Leu Tyr Gln Glu Ser Leu Leu Leu Ser Asn Leu
20 25 30

Asp Asp Ser Phe Ser Ala Asp Glu Thr Gly Asp Ser Asn Asp Pro Glu
35 40 45

Gln Ile Phe Gln Asn Ile Gln Phe Gln Lys Asp Leu Met Ala Asn Ile
50 55 60

Arg Cys Arg Pro Trp Thr Met Gly Gln Lys Leu Arg Ala Leu Arg Gln
65 70 75 80

Ala Lys Asn Ile Val Leu Lys Phe Glu Gly Arg Leu Thr Arg Thr Arg
85 90 95

Gly Tyr Gln Ala Ala Gly Ala Glu Leu Trp Arg Lys Phe Ala Arg Leu
100 105 110

Ala Cys Asn Phe Val Val Ile Phe Ile Pro Trp Glu Met Arg Ile Lys
115 120 125

Lys Ile Glu Ser His Phe Gly Ser Gly Val Ala Ser Tyr Phe Ile Phe
130 135 140

Leu Arg Trp Leu Phe Gly Ile Asn Ile Val Leu Thr Ile Met Thr Gly
145 150 155 160

Ala Phe Ile Val Ile Pro Glu Leu Ile Ala Gly Gln Pro Phe Gly Ser
165 170 175

Thr Ala Arg Lys Thr Ile Pro Lys Glu Gln Val Ser Ser Ala Gln Asp
180 185 190

Leu Asp Thr Val Trp Ser Leu Gly Gly Tyr Leu Gln Tyr Ser Val Leu
195 200 205

Phe Tyr Gly Tyr Tyr Gly Arg Glu Arg Lys Ile Gly Arg Ala Gly Tyr
210 215 220

Arg Leu Pro Leu Ala Tyr Phe Leu Val Gly Met Ala Val Phe Ala Tyr
225 230 235 240

Ser Phe Ile Ile Leu Leu Lys Lys Met Ala Lys Asn Ser Arg Thr Ser
245 250 255

Leu Ala Ser Ala Ser Asn Glu Asn Tyr Thr Phe Cys Trp Arg Val Phe
260 265 270

Cys Ala Trp Asp Tyr Leu Ile Gly Asn Pro Glu Ala Ala Glu Ser Lys
275 280 285

-continued

Thr Ala Ala Ile Val Asn Ser Ile
290 295

<210> SEQ ID NO 13
<211> LENGTH: 1174
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 13

cctgagcacc agatgcaggc agacatttgc tcctgtttct gaaggaggag agcaggtcaa 60

ctctgcaggc ctctttcaga cagctcattt ggaacagaac cctgcctgca aaatcaaggc 120

tttccaattc cctggctatc cctagggacc attggctaca gaccaggcct catgaaaacc 180

tcaaaggcat cccagcgcta cagaagcatt cggagaaacg ccagccagtg ttatctctac 240

caggactccc tgcttcttgg taactccgat gacagcttta atgctgatga aacgggagac 300

agcagtgatc cggaacaaat cttccagaat atacagtttc agaaagatct catggcaaat 360

atccgctgca gaccctggac catggggcag aaactgcggg cactgagacg agccaaggag 420

attgtgctga agtttgaagg gaggctgacc aggactcgag gctaccaggc agcaggagca 480

gagctctggc ggaaattcgc ccgtctggcc tgtaactttg tggtcatctt catcccctgg 540

gaaatgagga taaagaaaat tgagagtcac ttcgggtctg gtgtggcctc ctacttcatc 600

ttcctgaggt ggctatttgg gattaacata gtgctcacag tgatgaccgg tgcctttgtt 660

gtccttcccg agctgatcgc aggccagccc tttggaagta cagccagcaa gaccattccc 720

cgggagcaga tcacatctgc acaggatctg gacactgtct ggtccctggg gggctacctt 780

caatactccg tcctgttcta cgggtactac ggaagagaga ggcggatcgg gagagccggc 840

taccgcctgc ccttggcgta tttcctggtg gggatggcag tgtttgctta cagcttcatc 900

gttctcttaa aaaagatggc taagaactcc cgcaccagcc tagccagtgc ttccaatgaa 960

aattacacct tctgctggcg ggtgttttgt gcctgggatt atctcattgg gaacccagag 1020

gctgcagaaa gcaaaacagc tgccatcttg aacagcatta ggggcaacag aggcaaagag 1080

gtgaccatgg ttgataatga aagccaagag cccaacgaat gtgggaacaa acgctcaaaa 1140

cgtcttattt ctgtgtagtg ttccattgtc ttaa 1174

<210> SEQ ID NO 14
<211> LENGTH: 328
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 14

Met Lys Thr Ser Lys Ala Ser Gln Arg Tyr Arg Ser Ile Arg Arg Asn
1 5 10 15

Ala Ser Gln Cys Tyr Leu Tyr Gln Asp Ser Leu Leu Leu Gly Asn Ser
20 25 30

Asp Asp Ser Phe Asn Ala Asp Glu Thr Gly Asp Ser Ser Asp Pro Glu
35 40 45

Gln Ile Phe Gln Asn Ile Gln Phe Gln Lys Asp Leu Met Ala Asn Ile
50 55 60

Arg Cys Arg Pro Trp Thr Met Gly Gln Lys Leu Arg Ala Leu Arg Arg
65 70 75 80

Ala Lys Glu Ile Val Leu Lys Phe Glu Gly Arg Leu Thr Arg Thr Arg

-continued

```
                85                  90                  95

Gly Tyr Gln Ala Ala Gly Ala Glu Leu Trp Arg Lys Phe Ala Arg Leu
            100                 105                 110

Ala Cys Asn Phe Val Val Ile Phe Ile Pro Trp Glu Met Arg Ile Lys
        115                 120                 125

Lys Ile Glu Ser His Phe Gly Ser Gly Val Ala Ser Tyr Phe Ile Phe
    130                 135                 140

Leu Arg Trp Leu Phe Gly Ile Asn Ile Val Leu Thr Val Met Thr Gly
145                 150                 155                 160

Ala Phe Val Val Leu Pro Glu Leu Ile Ala Gly Gln Pro Phe Gly Ser
                165                 170                 175

Thr Ala Ser Lys Thr Ile Pro Arg Glu Gln Ile Thr Ser Ala Gln Asp
            180                 185                 190

Leu Asp Thr Val Trp Ser Leu Gly Gly Tyr Leu Gln Tyr Ser Val Leu
        195                 200                 205

Phe Tyr Gly Tyr Tyr Gly Arg Glu Arg Arg Ile Gly Arg Ala Gly Tyr
    210                 215                 220

Arg Leu Pro Leu Ala Tyr Phe Leu Val Gly Met Ala Val Phe Ala Tyr
225                 230                 235                 240

Ser Phe Ile Val Leu Leu Lys Lys Met Ala Lys Asn Ser Arg Thr Ser
                245                 250                 255

Leu Ala Ser Ala Ser Asn Glu Asn Tyr Thr Phe Cys Trp Arg Val Phe
            260                 265                 270

Cys Ala Trp Asp Tyr Leu Ile Gly Asn Pro Glu Ala Ala Glu Ser Lys
        275                 280                 285

Thr Ala Ala Ile Leu Asn Ser Ile Arg Gly Asn Arg Gly Lys Glu Val
    290                 295                 300

Thr Met Val Asp Asn Glu Ser Gln Glu Pro Asn Glu Cys Gly Asn Lys
305                 310                 315                 320

Arg Ser Lys Arg Leu Ile Ser Val
                325
```

<210> SEQ ID NO 15
<211> LENGTH: 822
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

```
cagcgtggcc ttcggcaagt gactaaaggg cttcctgcct cagctttccc cccagtccca     60

ggggcagggg gcagtgggcc cacagtggag ggcgaggctc ccgggctctt tctgtccagc    120

caggagcaga gagcgagaga cactgagggg cccagggtag ccctgtccca gcttcagtgc    180

gggctgctgg gctctgcaga acagtccttc ctgcagctgg agcaggagaa ccacagcctg    240

aaaaggcaga atcaggaact tcgggagcag ctgggggccc tcctggggcc ggggcagcag    300

ttcctgcccc tgtgtcccga acactcaagc tgcactgctc tggcctgggt acccccccgac    360

ccggctggca cgcagccctt ggggaacagg gcacctctgc agctgctgcg gcgggagctg    420

tgccaggggc aagaggcttt cgtgcaacag tccaacgagc tgcagcagat ccgcctgtgc    480

tttgagagga agaagatggt catcacagag gtgtgggaca acgtggctga gatgcacatg    540

gccctgaaca accaggccac cgggctcctg aacctcaaga aggacatccg gggcgtgctg    600

gaccagatgg aggacatcca gctggagatt ctcaggctgc tgaccctgag gctgctgctg    660
```

-continued

ggcgccctgc tggtctggac cgctgcctac gtgtacgtgg tgaaccccac acctttcgag 720

gggctggtgc caccctgct gagccgtgcc accgtctgga agctccgggc cctgctggac 780

cccttcctgc gcctcaaagt ggacggcttc ctgcccttct ag 822

<210> SEQ ID NO 16
<211> LENGTH: 273
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 16

Gln Arg Gly Leu Arg Gln Val Thr Lys Gly Leu Pro Ala Ser Ala Phe
1 5 10 15

Pro Pro Val Pro Gly Ala Gly Gly Ser Gly Pro Thr Val Glu Gly Glu
20 25 30

Ala Pro Gly Leu Phe Leu Ser Ser Gln Glu Gln Arg Ala Arg Asp Thr
35 40 45

Glu Gly Pro Arg Val Ala Leu Ser Gln Leu Gln Cys Gly Leu Leu Gly
50 55 60

Ser Ala Glu Gln Ser Phe Leu Gln Leu Glu Gln Glu Asn His Ser Leu
65 70 75 80

Lys Arg Gln Asn Gln Glu Leu Arg Glu Gln Leu Gly Ala Leu Leu Gly
85 90 95

Pro Gly Gln Gln Phe Leu Pro Leu Cys Pro Glu His Ser Ser Cys Thr
100 105 110

Ala Leu Ala Trp Val Pro Pro Asp Pro Ala Gly Thr Gln Pro Leu Gly
115 120 125

Asn Arg Ala Pro Leu Gln Leu Leu Arg Arg Glu Leu Cys Gln Gly Gln
130 135 140

Glu Ala Phe Val Gln Gln Ser Asn Glu Leu Gln Gln Ile Arg Leu Cys
145 150 155 160

Phe Glu Arg Lys Lys Met Val Ile Thr Glu Val Trp Asp Asn Val Ala
165 170 175

Glu Met His Met Ala Leu Asn Asn Gln Ala Thr Gly Leu Leu Asn Leu
180 185 190

Lys Lys Asp Ile Arg Gly Val Leu Asp Gln Met Glu Asp Ile Gln Leu
195 200 205

Glu Ile Leu Arg Leu Leu Thr Leu Arg Leu Leu Leu Gly Ala Leu Leu
210 215 220

Val Trp Thr Ala Ala Tyr Val Tyr Val Val Asn Pro Thr Pro Phe Glu
225 230 235 240

Gly Leu Val Pro Pro Leu Leu Ser Arg Ala Thr Val Trp Lys Leu Arg
245 250 255

Ala Leu Leu Asp Pro Phe Leu Arg Leu Lys Val Asp Gly Phe Leu Pro
260 265 270

Phe

<210> SEQ ID NO 17
<211> LENGTH: 831
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

-continued

```
cagcgtggcc ttcggcaagt gactaaaggg cttcctgcct cagcttcccc cccagtccca     60

ggggcagggg gtagtgggga ggggatccag ggtggagggc gaggctgcca ggatcttttc    120

tgtccagcga ggagcagaga gcgagaaacc ctgaagggcc ccaaggcagc cctgtcccag    180

cttcagtgtg ggcctctggg ctctgcagaa cagtccttcc tggagcagga gaaccacagc    240

ctgaaaagac agaaccagga ccttcgggag cagctggggg ccctcctggg gccggggcag    300

cagttcctgc cctgtgtccc gaacactcaa gctgcactgc tctggcctgg gccccccgac    360

ccggctggca cgcagccctt ggggaacagg gcacctctgc agctgctgcg gcgggagctg    420

tcccaggggc aagaggcttt cgtgcagcag aacgagctgc agcagatccg cctgtgcttt    480

gagaggaaga agatggtcat cacagagcgt gaccccacct tctcccaggc gtgggacaac    540

gtggctgaga tgcacgtggc cctgaacaac caggccaccg ggctcctgaa cctcaagaag    600

gacatctggg gcgtgctgga ccagatggag gacatccagc tggagattct caggctgctg    660

atcctgaggc tgctgctggg caccctgctg gtcgcctacg tgtacatggt gaaccccagg    720

cccttcgagg ggctggtgcc gcccctgctg agccgtgcca ccatctggaa gctccgggcc    780

ctgctggacc ccttcctgcg ccttgaggtg gatggcttcc tgtccttcta g              831
```

<210> SEQ ID NO 18
<211> LENGTH: 276
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

```
Gln Arg Gly Leu Arg Gln Val Thr Lys Gly Leu Pro Ala Ser Ala Ser
1               5                   10                  15

Pro Pro Val Pro Gly Ala Gly Gly Ser Gly Glu Gly Ile Gln Gly Gly
            20                  25                  30

Gly Arg Gly Cys Gln Asp Leu Phe Cys Pro Ala Arg Ser Arg Glu Arg
        35                  40                  45

Glu Thr Leu Lys Gly Pro Lys Ala Ala Leu Ser Gln Leu Gln Cys Gly
    50                  55                  60

Pro Leu Gly Ser Ala Glu Gln Ser Phe Leu Glu Gln Glu Asn His Ser
65                  70                  75                  80

Leu Lys Arg Gln Asn Gln Asp Leu Arg Glu Gln Leu Gly Ala Leu Leu
                85                  90                  95

Gly Pro Gly Gln Gln Phe Leu Pro Cys Val Pro Asn Thr Gln Ala Ala
            100                 105                 110

Leu Leu Trp Pro Gly Pro Pro Asp Pro Ala Gly Thr Gln Pro Leu Gly
        115                 120                 125

Asn Arg Ala Pro Leu Gln Leu Leu Arg Arg Glu Leu Ser Gln Gly Gln
    130                 135                 140

Glu Ala Phe Val Gln Gln Asn Glu Leu Gln Gln Ile Arg Leu Cys Phe
145                 150                 155                 160

Glu Arg Lys Lys Met Val Ile Thr Glu Arg Asp Pro Thr Phe Ser Gln
                165                 170                 175

Ala Trp Asp Asn Val Ala Glu Met His Val Ala Leu Asn Asn Gln Ala
            180                 185                 190

Thr Gly Leu Leu Asn Leu Lys Lys Asp Ile Trp Gly Val Leu Asp Gln
        195                 200                 205

Met Glu Asp Ile Gln Leu Glu Ile Leu Arg Leu Leu Ile Leu Arg Leu
```

-continued

```
    210                 215                 220

Leu Leu Gly Thr Leu Leu Val Ala Tyr Val Tyr Met Val Asn Pro Arg
225                 230                 235                 240

Pro Phe Glu Gly Leu Val Pro Pro Leu Leu Ser Arg Ala Thr Ile Trp
                245                 250                 255

Lys Leu Arg Ala Leu Leu Asp Pro Phe Leu Arg Leu Glu Val Asp Gly
            260                 265                 270

Phe Leu Ser Phe
        275
```

<210> SEQ ID NO 19
<211> LENGTH: 609
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 19

```
ggcacagccc aacaaagagg aaaggccaca gcttcatcta gaccagctcc cacacagctt     60

cagggcagga cactggggtc tgcagaacaa tcctttctgc agctggagca ggagaaccag    120

agtctgagtg agctgcaaca gatccgattg tcctttgaga ggaagaagat ggccattact    180

gaggtgtggg atggtgtggc cgaagtacac atggctctga acaaccaagc caccgggctc    240

ctgaacctca agaaggacat ccggggtgtg ttagagcaga tggaagacat tcagctggag    300

atactggggg agagggccca ttgccgcact caggccagga agcaacagca aatgatggag    360

aaaggcaggc cacagatggg atgttccgag ggcctaaagg gccacctctg gctgctggcc    420

ttgaggctgc tgctgggtgc tctgctggct cgcacagcgg cctacgtata cgtggtggac    480

cccacaccct tcgaggggct ggtaccaccc ctgctgagca gagctgctgt ctggaagctc    540

agggccctac tgggcccctt cttgcgcctc gaagtggacg acttcctgcc tttctagacc    600

ggaagccca                                                            609
```

<210> SEQ ID NO 20
<211> LENGTH: 198
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 20

```
Gly Thr Ala Gln Gln Arg Gly Lys Ala Thr Ala Ser Ser Arg Pro Ala
1               5                   10                  15

Pro Thr Gln Leu Gln Gly Arg Thr Leu Gly Ser Ala Glu Gln Ser Phe
            20                  25                  30

Leu Gln Leu Glu Gln Glu Asn Gln Ser Leu Ser Glu Leu Gln Gln Ile
        35                  40                  45

Arg Leu Ser Phe Glu Arg Lys Lys Met Ala Ile Thr Glu Val Trp Asp
    50                  55                  60

Gly Val Ala Glu Val His Met Ala Leu Asn Asn Gln Ala Thr Gly Leu
65                  70                  75                  80

Leu Asn Leu Lys Lys Asp Ile Arg Gly Val Leu Glu Gln Met Glu Asp
                85                  90                  95

Ile Gln Leu Glu Ile Leu Gly Glu Arg Ala His Cys Arg Thr Gln Ala
            100                 105                 110

Arg Lys Gln Gln Gln Met Met Glu Lys Gly Arg Pro Gln Met Gly Cys
        115                 120                 125
```

-continued

```
Ser Glu Gly Leu Lys Gly His Leu Trp Leu Leu Ala Leu Arg Leu Leu
    130                 135                 140

Leu Gly Ala Leu Leu Ala Arg Thr Ala Ala Tyr Val Tyr Val Val Asp
145                 150                 155                 160

Pro Thr Pro Phe Glu Gly Leu Val Pro Pro Leu Leu Ser Arg Ala Ala
                165                 170                 175

Val Trp Lys Leu Arg Ala Leu Leu Gly Pro Phe Leu Arg Leu Glu Val
            180                 185                 190

Asp Asp Phe Leu Pro Phe
        195
```

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:

(a) SEQ ID NO:12;

(b) SEQ ID NO:16 or SEQ ID NO:18;

(c) a fragment of the amino acid sequences of (a) or (b) comprising at least 20 contiguous amino acids and having immunomodulatory activity;

(d) a fragment of the amino acid sequences of (a) or (b) comprising at least 30 contiguous amino acids and having immunomodulatory activity;

(f) a fragment of the amino acid sequences of any of (a) or (b) comprising alpha helix amino acid sequences;

(h) amino acid sequences comprising at least 20 amino acids and sharing amino acid identity with the amino acid sequences of any of (a)-(g), wherein the percent amino acid identity is selected from the group consisting of: at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97.5%, at least 99%, and at least 99.5%;

2. The polypeptide of claim 1, wherein the polypeptide has immunomodulatory activity.

3. An isolated nucleic acid encoding a polypeptide of claim 1.

4. An isolated genomic nucleic acid corresponding to the nucleic acid of claim 3.

5. An isolated nucleic acid, having a length of at least 15 nucleotides, that hybridizes under conditions of moderate stringency to the nucleic acid of claim 3 and encodes a polypeptide having immunomodulatory activity.

6. An isolated nucleic acid comprising a nucleotide sequence that encodes a polypeptide having immunomodulatory activity and shares nucleotide sequence identity with the nucleotide sequences of the nucleic acids of claim 3, wherein the percent nucleotide sequence identity is selected from the group consisting of: at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 97.5%, at least 99%, and at least 99.5%.

7. An expression vector comprising at least one nucleic acid according to claim 3.

8. A recombinant host cell comprising at least one nucleic acid according to claim 3.

9. The recombinant host cell of claim 8, wherein the nucleic acid is integrated into the host cell genome.

10. A process for producing a polypeptide encoded by the nucleic acid of claim 3, comprising culturing a recombinant host cell under conditions promoting expression of said polypeptide, wherein the recombinant host cell comprises at least one nucleic acid of claim 3.

11. The process of claim 10 further comprising purifying said polypeptide.

12. The polypeptide produced by the process of claim 10.

13. An isolated antibody that binds to the polypeptide of any of claim 12.

14. The antibody of claim 13 wherein the antibody is a human antibody.

15. The antibody of claim 13 wherein the antibody inhibits the activity of the polypeptide of claim 12.

16. A method for identifying compounds that alter immunomodulatory activity comprising

(a) mixing a test compound with the polypeptide of claim 12; and

(b) determining whether the test compound alters the immunomodulatory activity of said polypeptide.

17. A method for identifying compounds that inhibit the binding activity of alpha-helix-containing polypeptides comprising

(a) mixing a test compound with the polypeptide of claim 12 and a binding partner of said polypeptide; and

(b) determining whether the test compound inhibits the binding activity of said polypeptide.

18. A method for increasing the proliferation or the development of cells from pluripotent stem cell precursors comprising providing at least one compound selected from the group consisting of the polypeptide of claim 12 and agonists of said polypeptides.

19. A method for decreasing the proliferation or the development of cells from pluripotent stem cell precursors comprising providing at least one antagonist of the polypeptide of claim 12.

20. The method of claim **23** wherein the antagonist is an antibody that inhibits the activity of said polypeptide.

* * * * *