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(54) Titre : POLYPEPTIDES DES CYTOKINES HUMAINE ET MURINE
(54) Title: HUMAN AND MURINE CYTOKINE POLYPEPTIDES

(57) Abrégé/Abstract:
This invention relates to IMX7189 cytokines and to new members of the human cytokine polypeptide family, methods of making such polypeptides, and to methods of using them to treat conditions and diseases involving proliferation and/or differentiation of cells from pluripotent stem cell precursors, and to identify compounds that alter cytokine polypeptide activities.

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(54) Title: HUMAN AND MURINE CYTOKINE POLYPEPTIDES

(57) Abstract: This invention relates to IMX7189 cytokines and to new members of the human cytokine polypeptide family, methods of making such polypeptides, and to methods of using them to treat conditions and diseases involving proliferation and/or differentiation of cells from pluripotent stem cell precursors, and to identify compounds that alter cytokine polypeptide activities.

HUMAN AND MURINE CYTOKINE POLYPEPTIDES

This application claims the benefit under 35 U.S.C. 119(e) of U.S. provisional application Serial No. 60/327,122, filed 03 October 2001, which is incorporated in its entirety by reference herein.

FIELD OF THE INVENTION

This invention relates to IMX7189 cytokine polypeptides, such as human and murine IMX7189 polypeptides; to novel human polypeptides having structural similarity to "four-alpha-helical-bundle" (4AHB) cytokines; and to methods of making and using these IMX7189 cytokine polypeptides and novel human cytokines of the invention.

BACKGROUND OF THE INVENTION

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).

Common structural features of the 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 (Grötzinger *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.

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 II 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).

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) or Th2 cell fates (IL-2, IL-4, and IL-15, for example) (Nicola and Hilton, 1999, *Advances in Protein Chemistry* 52: 1-65).

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.

SUMMARY OF THE INVENTION

The present invention is based upon the discovery that human IMX7189 polypeptide is a 4AHB cytokine, and upon the discoveries of a murine IMX7189 cytokine polypeptide and additional novel human polypeptides having structures similar to 4AHB cytokines.

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:

(a) the amino acid sequence of SEQ ID NO:2;

(b) the amino acid sequence of SEQ ID NO:5;

(c) an amino acid sequence that begins between amino acid A through B and ends between amino acid Y through Z, wherein sets of values for A, B, Y, and Z are selected from the group consisting of: A = 35, B = 40, Y = 52, and Z = 53 of SEQ ID NO:2 or of SEQ ID NO:5; A = 72, B = 74, Y = 95, and Z = 98 of SEQ ID NO:2 or of SEQ ID NO:5; A = 98, B = 101, Y = 122, and Z = 123 of SEQ ID NO:2 or of SEQ ID NO:5; and A = 123, B = 124, Y = 136, and Z = 139 of SEQ NO:2 or of SEQ ID NO:5;

(d) a fragment of an amino acid sequence of any of (a)-(c) comprising at least 20 contiguous amino acids;

(e) a fragment of an amino acid sequence of any of (a)-(c) comprising at least 30 contiguous amino acids;

(f) a fragment of an amino acid sequence of any of (a)-(c) having cytokine polypeptide activity;

(g) a fragment of an amino acid sequence of any of (a)-(c) comprising Helix A and/ or Helix D 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 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%;

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

(j) an amino acid sequence of (h) or (i) having cytokine polypeptide activity;

wherein said isolated polypeptide does not consist of the amino acid sequence of any of the polypeptides disclosed in WO 00/70047 (GeneSeq AAB36627), WO 01/53312 (GeneSeq AAM40250); TrEMBL database accession numbers Q9BST1 and Q9NWKO, GenBank accession numbers AAH04818, XP_040852.1, and BAA91380.1, TrEMBL database accession number Q9P0R6, GenBank accession number NP_057556, WO 00/55171 (GeneSeq AAB28000), or WO 00/61620 (GeneSeq AAB51684).

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

(a) the amino acid sequence of SEQ ID NO:4;

(b) an amino acid sequence that begins between amino acid A through B and ends between amino acid Y through Z, wherein sets of values for A, B, Y, and Z are selected from the

group consisting of: A = 40, B = 45, Y = 57, and Z = 58 of SEQ ID NO:4; A = 77, B = 79, Y = 100, and Z = 103 of SEQ ID NO:4; A = 103, B = 106, Y = 127, and Z = 128 of SEQ ID NO:4; and A = 128, B = 129, Y = 141, and Z = 144 of SEQ NO:4;

(c) a fragment of an amino acid sequence of any of (a)-(b) comprising at least 20 contiguous amino acids;

(d) a fragment of an amino acid sequence of any of (a)-(b) comprising at least 30 contiguous amino acids;

(e) a fragment of an amino acid sequence of any of (a)-(b) having cytokine polypeptide activity;

(f) a fragment of an amino acid sequence of any of (a)-(b) comprising Helix A and/ or Helix D amino acid sequences;

(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%;

(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

(i) an amino acid sequence of (g) or (h) having cytokine polypeptide activity.

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

(a) an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9;

(b) an amino acid sequence that begins between amino acid A through B and ends between amino acid Y through Z, wherein sets of values for A, B, Y, and Z are selected from the group consisting of: A = 73, B = 94, Y = 108, and Z = 118 of SEQ ID NO:6; A = 153, B = 153, Y = 161, and Z = 173 of SEQ ID NO:6; A = 190, B = 191, Y = 212, and Z = 212 of SEQ NO:6; A = 213, B = 218, Y = 253, and Z = 254 of SEQ ID NO:6; A = 44, B = 47, Y = 82, and Z = 83 of SEQ ID NO:7; A = 114, B = 120, Y = 132, and Z = 152 of SEQ NO:7; A = 140, B = 165, Y = 176, and Z = 178 of SEQ ID NO:7; A = 195, B = 198, Y = 240, and Z = 243 of SEQ ID NO:7; A = 587, B = 588, Y = 613, and Z = 615 of SEQ NO:8; A = 639, B = 643, Y = 664, and Z = 669 of SEQ ID NO:8; A = 673, B = 674, Y = 700, and Z = 702 of SEQ ID NO:8; A = 715, B = 715, Y = 724, and Z = 730 of SEQ NO:8; A = 27, B = 29, Y = 39, and Z = 41 of SEQ NO:9; A = 66, B = 68, Y = 80, and Z = 101 of SEQ ID NO:9; A = 111, B = 111, Y = 133, and Z = 143 of SEQ ID NO:9; and A = 147, B = 148, Y = 177, and Z = 187 of SEQ NO:9;

(c) a fragment of an amino acid sequence of any of (a)-(b) comprising at least 20 contiguous amino acids;

(d) a fragment of an amino acid sequence of any of (a)-(b) comprising at least 30 contiguous amino acids;

(e) a fragment of an amino acid sequence of any of (a)-(b) having cytokine polypeptide activity;

(f) a fragment of an amino acid sequence of any of (a)-(b) comprising Helix A and/ or Helix D amino acid sequences;

(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%;

(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

(i) an amino acid sequence of (g) or (h) having cytokine polypeptide activity.

Other aspects of the invention are isolated nucleic acids encoding polypeptides of the invention, with a preferred embodiment being an isolated nucleic acid consisting of, consisting essentially of, or more preferably, comprising a nucleotide sequence selected from the group consisting of:

(a) nucleotides 203 through 619 SEQ ID NO:1;

(b) nucleotides 187 through 618 of SEQ ID NO:3;

(c) variants of (a)-(b).

An additional preferred embodiment of the invention is an isolated nucleic acid consisting of, consisting essentially of, or more preferably, comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:3.

The invention also provides an isolated genomic nucleic acid corresponding to the nucleic acids of the invention.

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 cytokine polypeptide 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%.

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.

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.

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.

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.

In another aspect of the invention, a method is provided for identifying peptide agonists and antagonists of the polypeptides of the invention, the method comprising selecting at least one peptide that binds to a polypeptide of the invention, wherein the peptide is selected in a process comprising one or more techniques selected from yeast-based screening, rational design, protein structural analysis, screening of a phage display library, an *E. coli* display library, a ribosomal library, an RNA-peptide library, and a chemical peptide library. In further aspects of the invention, the peptide is selected from a plurality of randomized peptides.

In a further aspect of the invention, a method is provided for identifying compounds that alter activities of the cytokine polypeptides of the invention comprising

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

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

In another aspect of the invention, a method is provided identifying compounds that inhibit the binding activity of cytokine polypeptides of the invention comprising

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

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

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.

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.

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.

The invention additionally provides a method for increasing the number of cytokine-receptor-bearing cells or their developmentally committed progeny, through increased cell proliferation and/ or altered cell differentiation, comprising contacting said cytokine-receptor-bearing cells with polypeptides of the invention or agonists thereof. In preferred embodiments, the cytokine-receptor-bearing cells are pluripotent cells, and in further preferred embodiments, the cytokine-receptor-bearing cells are cells of the hematopoietic system.

In other aspects of the invention, methods are provided for treating cytopenias for cytokine-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 cytokine-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.

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.

DETAILED DESCRIPTION OF THE INVENTION

Similarities of IMX7189 and Novel Human Cytokine Structures to Other 4AHB Cytokines

We have determined that a certain human protein is structurally related to human 4AHB cytokines and have identified a homologous murine 4AHB cytokine; these human and murine cytokines have been named IMX7189. The amino acid sequences of the human and murine cytokine polypeptides of the invention are provided in SEQ ID NOs 2 and 4, respectively, and an alignment showing the amino acid sequence similarities between these IMX7189 cytokines is presented in Table 1 in Example 1 below. Additional new human polypeptides that are also structurally related to 4AHB cytokines are provided in SEQ ID NOs 6 through 9. As used herein, "cytokine polypeptides of the

invention" refers to the group of polypeptides consisting of human and murine IMX7189 polypeptides (SEQ ID NOs 2 and 4) and the polypeptides of SEQ ID NOs 6 through 9.

The typical structural elements common to members of the 4AHB cytokine polypeptide family include four 'core' alpha helices separated by loops which are termed, in N-to-C order, helix A, loop AB, helix B, loop BC, helix C, loop CD, and helix D. The approximate locations of the four alpha helices in the IMX7189 cytokine polypeptide sequences (SEQ ID NOs 2 and 4) are shown in the table below. The locations of these helices within IMX7189 cytokine polypeptides were determined by using the GeneFold program (described in more detail in Example 1 below) to find the regions in IMX7189 polypeptides that fit most closely to the known alpha helices of cytokine template polypeptide structures such as IL-4. The locations of the alpha helices in four additional novel human polypeptides having structures similar to 4AHB cytokines are indicated in further tables below. Note that in some cases there is an overlap between the predicted extent of helix B and helix C; this can result 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. Therefore, cytokine polypeptides of the invention and the four additional novel human polypeptides disclosed herein have an overall four-helical structure consistent with that of other 4AHB cytokine polypeptides.

	Location of Alpha Helices							
	Human IMX7189 (SEQ ID NO:2)				Murine IMX7189 (SEQ ID NO:4)			
	Begins between:		Ends between:		Begins between		Ends between:	
	A	**B**	**Y**	**Z**	**A**	**B**	**Y**	**Z**
Helix A	35	40	52	53	40	45	57	58
Helix B	72	74	95	98	77	79	100	103
Helix C	98	101	122	123	103	106	127	128
Helix D	123	124	136	139	128	129	141	144
	IMX168745 (SEQ ID NO:6)				IMX185787 (SEQ ID NO:7)			
	Begins **A** - **B**:		Ends **Y** - **Z**:		Begins **A** - **B**:		Ends **Y** - **Z**:	
Helix	**A**	**B**	**Y**	**Z**	**A**	**B**	**Y**	**Z**
A	73	94	108	118	44	47	82	83
B	153	153	161	173	114	120	132	152
C	190	191	212	212	140	165	176	178
D	213	218	253	254	195	198	240	243
	IMX188339 (SEQ ID NO:8)				IMX192967 (SEQ ID NO:9)			
	Begins **A** - **B**:		Ends **Y** - **Z**:		Begins **A** - **B**:		Ends **Y** - **Z**:	
Helix	**A**	**B**	**Y**	**Z**	**A**	**B**	**Y**	**Z**
A	587	588	613	615	27	29	39	41
B	639	643	664	669	66	68	80	101
C	673	674	700	702	111	111	133	143
D	715	715	724	730	147	148	177	187

The human and murine IMX7189 cytokine polypeptides (SEQ ID NOs 2 and 4) are also similar in amino acid sequence to polypeptides from other species such as Caenorhabditis elegans ('ce') and Drosophila melanogaster ('dm'), as shown in Table 1 in Example 1 below. In addition to the sequences shown in Table 1, there are two more Caenorhabditis polypeptides (TrEMBL database accession numbers Q9XU41 and Q9XWX7) having amino acid sequence similarity to IMX7189 cytokine polypeptides. The biological function for these Caenorhabditis and Drosophila polypeptides had apparently not yet been determined. We have discovered that, based on the similarity in sequence between the human and murine IMX7189 cytokines and the Caenorhabditis and Drosophila polypeptides within the region aligning well with the alpha helices of IL-4, it is likely that the Caenorhabditis and Drosophila polypeptides are 4AHB polypeptides with functions analogous to mammalian cytokines. Even the most significant difference between the Drosophila Q9VNV2 polypeptide sequence (SEQ ID NO:13) and that of the others in Table 1 is consistent with Drosophila Q9VNV2 polypeptide being a 4AHB polypeptide: in Drosophila Q9VNV2 there is an insertion of approximately 11 amino acids between the amino acids corresponding to amino acids 99 and 100 of human IMX7189 polypeptide (SEQ ID NO:2), which places the insertion in the flexible BC loop region between helices B and C, making it less likely to disrupt the 4AHB structure of Drosophila Q9VNV2 polypeptide.

The skilled artisan will recognize that the boundaries of the domains of IMX7189 cytokine polypeptides and the novel human polypeptides described above are approximate, and that the precise boundaries of such domains, as for example the boundaries of the alpha helices (which can be predicted by using computer programs available for that purpose), can also differ from member to member within the 4AHB cytokine polypeptide family.

Members of the 4AHB cytokine family are secreted polypeptides and most, such as IMX168745 polypeptide.(SEQ ID NO:6), have signal sequences that are predicted on the basis of examination of the amino acid sequence. Although the human and murine IMX7189 cytokine polypeptides do not appear to have a canonical signal sequence, transient expression experiments in which COS cells were transfected with a pDC414G vector encoding human IMX7189 polypeptide indicate that human IMX7189 polypeptide was secreted from the transfected COS cells. Therefore, despite the absence of a discernable signal sequence, polypeptides such as human IMX7189 that are similar in structure to 4AHB cytokines can be secreted from cells.

Biological Activities and Functions of Cytokine Polypeptides of the Invention

Typical biological activities or functions associated with IMX7189 and the present novel human cytokine polypeptides are stimulation of the proliferation and/or stimulation of the differentiation of cells from pluripotent stem cell precursors. Cytokine polypeptides of the invention having stimulation of cell proliferation activity bind receptor polypeptides. The receptor-binding activity is associated with domains comprising helix A and helix D of cytokine polypeptides of the

invention. Thus, for uses requiring receptor-binding activity, preferred cytokine polypeptides of the invention include those having helix A and helix D and exhibiting stimulation of cell proliferation activity. Preferred cytokine polypeptides of the invention further include oligomers or fusion polypeptides comprising at least one alpha helix portion of one or more cytokine polypeptides of the invention, and fragments of any of these polypeptides that have stimulation of cell proliferation activity. The receptor-dependent stimulation of cell proliferation activity of cytokine polypeptides of the invention 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, Figure 6 of Kallen *et al.*, 1999, *J Biol Chem* 274: 11859-11867). Alternatively, the effect that treatment of cells with cytokine polypeptides of the invention 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, Figure 2 of Kallen *et al.*, 1999, *J Biol Chem* 274: 11859-11867). Cytokine polypeptides of the invention 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 Figure 6A of Kallen *et al.*, 1999, *J Biol Chem* 274: 11859-11867. Cytokine polypeptides of the invention 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 Figure 2A of Kallen *et al.*, 1999, *J Biol Chem* 274: 11859-11867. The term "cytokine polypeptide 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 cytokine polypeptides of the invention (for example, human and murine IMX7189). The degree to which individual cytokine polypeptides of the invention 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 the biological activities of cytokine polypeptides of the invention.

Another aspect of the biological activity of cytokine polypeptides of the invention is the ability of members of this polypeptide family to bind particular binding partners such as cell surface receptors that are members of the immunoglobulin superfamily, and more particularly to members of the cytokine receptor family. The term "binding partner," as used herein, includes ligands, receptors, substrates, antibodies, other cytokine polypeptides of the invention, the same cytokine polypeptide of the invention (in the case of homotypic interactions or formation of multimers), and any other molecule that interacts with a cytokine polypeptide of the invention through contact or proximity between particular portions of the binding partner and the cytokine polypeptide. Because helix A and helix D of cytokine polypeptides of the invention are likely to be involved in the cytokine-receptor interaction,

mutations of hydrophobic or charged residues within these helices are expected to alter the binding of cytokine polypeptides of the invention to receptor polypeptides; such mutations are likely to disrupt cytokine-receptor binding but may increase the strength of this interaction. By binding to one or more components of a cytokine receptor complex, or by binding to some components but not others, an altered cytokine polypeptide of the invention would likely prevent binding by the native cytokine polypeptide of the invention(s), and so act in a dominant negative fashion to inhibit the biological activities mediated via binding of cytokine polypeptides of the invention to cytokine receptors (see, for example, Tables I and II of interactions (Grötzinger *et al.*, 1997, *PROTEINS: Structure, Function, and Genetics* 27: 96-109). Suitable assays to detect or measure the binding between cytokine polypeptides of the invention and their binding partners are well known to those of skill in the art and are described herein.

Cytokine polypeptides of the invention 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 cytokine polypeptides of the invention 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 the activities of cytokine polypeptides of the invention. 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 cytokine polypeptides of the invention by providing isolated cytokine polypeptides of the invention or active fragments or fusion polypeptides thereof, or by providing compounds (agonists) that activate endogenous or exogenous cytokine polypeptides of the invention. Additional uses for cytokine polypeptides of the invention 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 cytokine receptors.

Cytokine Polypeptides of the Invention

A cytokine polypeptide of the invention is a polypeptide that shares a sufficient degree of amino acid identity or similarity to a polypeptide of SEQ ID NOs 2, 4, and 6 through 9 to (A) be identified by those of skill in the art as a polypeptide likely to share particular structural domains and/or (B) have biological activities in common with the cytokine polypeptide of SEQ ID NOs 2, 4, and 6 through 9 and/or (C) bind to antibodies that also specifically bind to other cytokine polypeptides of the invention. Cytokine polypeptides of the invention can be isolated from naturally occurring sources, or have the same structure as naturally occurring cytokine polypeptides of the invention, or can be

produced to have structures that differ from naturally occurring cytokine polypeptides of the invention. Polypeptides derived from any cytokine polypeptide of the invention 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 cytokine polypeptides of the invention. Therefore, the polypeptides provided by the invention include polypeptides characterized by amino acid sequences similar to those of the cytokine polypeptides of the invention described herein, but into which modifications are naturally provided or deliberately engineered. A polypeptide that shares biological activities in common with cytokine polypeptides of the invention is a polypeptide having cytokine polypeptide activity. Examples of biological activities exhibited by cytokine polypeptides of the invention include, without limitation, stimulation of proliferation and/or differentiation of cells from pluripotent stem cell precursors.

The present invention provides both full-length and mature forms of cytokine polypeptides of the invention. 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 can 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 can 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 cytokine polypeptides of the invention 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).

The invention further includes cytokine polypeptides of the invention 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).

Species homologues of cytokine polypeptides of the invention 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 can 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 cytokine polypeptides of the invention 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).

Fragments of the cytokine polypeptides of the invention of the present invention are encompassed by the present invention and can be in linear form or cyclized using known methods, for example, as described in Saragovi et al., Bio/Technology 10, 773-778 (1992) and in 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 cytokine polypeptide of the invention 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 cytokine 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 cytokine polypeptide of the invention, 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 of two amino acid or two nucleic acid sequences can be determined by visual inspection and mathematical calculation, or more preferably, the comparison is done by comparing sequence information using a computer program. An exemplary, preferred

computer program is the Genetics Computer Group (GCG; Madison, WI) Wisconsin package version 10.0 program, 'GAP' (Devereux *et al.*, 1984, *Nucl. Acids Res.* 12: 387). The preferred default parameters for the 'GAP' program includes: (1) The GCG implementation of a unary comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted amino acid comparison matrix of Gribskov and Burgess, *Nucl. Acids Res. 14*:6745, 1986, as described by Schwartz and Dayhoff, eds., *Atlas of Polypeptide Sequence and Structure*, National Biomedical Research Foundation, pp. 353-358, 1979; or other comparable comparison matrices; (2) a penalty of 30 for each gap and an additional penalty of 1 for each symbol in each gap for amino acid sequences, or penalty of 50 for each gap and an additional penalty of 3 for each symbol in each gap for nucleotide sequences; (3) no penalty for end gaps; and (4) no maximum penalty for long gaps. Other programs used by those skilled in the art of sequence comparison can also be used, such as, for example, the BLASTN program version 2.0.9, available for use *via* the National Library of Medicine website www.ncbi.nlm.nih.gov/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 site: sapiens.wustl.edu/blast/blast/#Features. In addition, the BLAST algorithm uses the BLOSUM62 amino acid scoring matix, and optional parameters that can be used are as follows: (A) inclusion of a filter to mask segments of the query sequence that have low compositional complexity (as determined by the SEG program of Wootton and Federhen (Computers and Chemistry, 1993); also see Wootton and Federhen, 1996, Analysis of compositionally biased regions in sequence databases, *Methods Enzymol.* 266: 554-71) or segments consisting of short-periodicity internal repeats (as determined by the XNU program of Claverie and States (Computers and Chemistry, 1993)), and (B) a statistical significance threshold for reporting matches against database sequences, or E-score (the expected probability of matches being found merely by chance, according to the stochastic model of Karlin and Altschul (1990); if the statistical significance ascribed to a match is greater than this E-score threshold, the match will not be reported.); preferred E-score threshold values are 0.5, or in order of increasing preference, 0.25, 0.1, 0.05, 0.01, 0.001, 0.0001, 1e-5, 1e-10, 1e-15, 1e-20, 1e-25, 1e-30, 1e-40, 1e-50, 1e-75, or 1e-100.

The present invention also provides for soluble forms of cytokine polypeptides of the invention comprising certain fragments or domains of these polypeptides. Soluble polypeptides are polypeptides that are capable of being secreted from the cells in which they are expressed. A secreted soluble polypeptide can 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 cytokine polypeptides of the invention 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.

"An isolated polypeptide consisting essentially of an amino acid sequence" means that the polypeptide may have, in addition to said amino acid sequence, additional material covalently linked to either or both ends of the polypeptide, said additional material preferably between 1 and 10,000 additional amino acids covalently linked to either end, each end, or both ends of polypeptide, and more preferably between 1 and 1,000 additional amino acids covalently linked to either end, each end, or both ends of the polypeptide, and most preferably between 1 and 100 additional amino acids covalently linked to either end, each end, or both ends of the polypeptide. In preferred embodiments, covalent linkage of additional amino acids to either end, each end, or both ends of the polypeptide results in a novel combined amino acid sequence that is neither naturally occuring nor disclosed in the art.

In another aspect of the invention, preferred polypeptides comprise various combinations of structures of cytokine polypeptides of the invention, such as helices A, B, C, and D and/or the inter-helix loops AB, BC, and CD. Accordingly, polypeptides of the present invention and nucleic acids encoding them include those comprising or encoding two or more copies of helix A, two or more copies of helix D, or at least one copy of each. A further embodiment of the invention is an isolated IMX7189 polypeptide consisting of the following, in N-to-C order: a polypeptide consisting essentially of helix A, covalently linked to a polypeptide consisting essentially of helix B, covalently linked to a polypeptide consisting essentially of helix C, covalently linked to a polypeptide consisting essentially of helix D, wherein a polypeptide consisting essentially of a given helix of the IMX7189 polypeptide may include a naturally occuring or a modified inter-helix loop amino acid sequence, for example, an inter-helix loop sequence in which conservative substitutions have been made of one or more amino acids. Isolated IMX7189 polypeptides of the invention specifically do not consist of the amino acid sequence of the polypeptides disclosed in WO 00/70047 (GeneSeq AAB36627), WO 01/53312 (GeneSeq AAM40250), TrEMBL database accession numbers Q9BST1 and Q9NWKO, GenBank accession numbers XP_040852.1 and BAA91380.1, TrEMBL database accession number Q9P0R6, GenBank accession number NP_057556, WO 00/55171 (GeneSeq AAB28000), or WO 00/61620 (GeneSeq AAB51684).

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 can include the alteration, substitution, replacement, insertion or deletion of a selected amino acid. For example, one or more of the cysteine residues can be deleted or replaced with another amino acid to alter the conformation of the molecule, an alteration which may involve preventing formation of incorrect intramolecular disulfide bridges upon folding or renaturation. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, e.g., U.S. Pat. No. 4,518,584). As another example, N-glycosylation sites in the polypeptide 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. Patent 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. Patent No. 5,512,457 and as set forth herein.

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. Patent 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. Patent 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, Connecticut.

Encompassed by the invention are oligomers or fusion polypeptides that contain a cytokine polypeptide of the invention, one or more fragments of cytokine polypeptides of the invention, or any of the derivative or variant forms of cytokine polypeptides of the invention as disclosed herein. In particular embodiments, the oligomers comprise soluble cytokine polypeptides of the invention. Oligomers 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 cytokine polypeptides of the invention 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.

In embodiments where variants of the cytokine polypeptides of the invention are constructed to include a membrane-spanning domain, they will form a Type I membrane polypeptide. Membrane-spanning cytokine polypeptides of the invention 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 cytokine polypeptide of the invention. Cytokine polypeptides of the invention that span the cell membrane can also be fused with agonists or antagonists of cell-surface receptors, or cellular adhesion molecules to further modulate the cytokine's intracellular effects. In another aspect of the present invention, other interleukin or cytokine polypeptides can be situated between the preferred fragment of the cytokine polypeptide of the invention and other fusion polypeptide domains.

Immunoglobulin-based Oligomers. The polypeptides of the invention or fragments thereof can be fused to molecules such as immunoglobulins for many purposes, including increasing the valency of polypeptide binding sites. For example, fragments of a cytokine polypeptide of the invention can 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 can also be used to generate such fusions. For example, a polypeptide-IgM fusion would generate a decavalent form of the polypeptide of the invention. 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. Patent 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 cytokine extracellular regions.

Peptide-linker Based Oligomers. Alternatively, the oligomer is a fusion polypeptide comprising multiple cytokine polypeptides of the invention, with or without peptide linkers (spacer peptides). Among the suitable peptide linkers are those described in U.S. Patents 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 cytokine polypeptides of the invention, separated by peptide linkers. Suitable peptide linkers, their combination with other polypeptides, and their use are well known by those skilled in the art.

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.

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 can 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 Cytokine Polypeptides of the Invention

Encompassed within the invention are nucleic acids encoding cytokine polypeptides of the invention. 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 cytokine polypeptide of the invention or a desired combination of fragments of the cytokine polypeptides of the invention. 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).

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.

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 nucleic acids are substantially free from contaminating endogenous 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 ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (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.

"An isolated nucleic acid consisting essentially of a nucleotide sequence" means that the nucleic acid may have, in addition to said nucleotide sequence, additional material covalently linked to

either or both ends of the nucleic acid molecule, said additional material preferably between 1 and 100,000 additional nucleotides covalently linked to either end, each end, or both ends of the nucleic acid molecule, and more preferably between 1 and 1,000 additional nucleotides covalently linked to either end, each end, or both ends of the nucleic acid molecule, and most preferably between 10 and 100 additional nucleotides covalently linked to either end, each end, or both ends of the nucleic acid molecule. In preferred embodiments, covalent linkage of additional nucleotides to either end, each end, or both ends of the nucleic acid molecule results in a novel combined nucleotide sequence that is neither naturally occuring nor disclosed in the art. An isolated nucleic acid consisting essentially of a nucleotide sequence may be an expression vector or other construct comprising said nucleotide sequence.

The present invention also includes nucleic acids that hybridize under moderately stringent conditions, and more preferably highly stringent conditions, to nucleic acids encoding cytokine polypeptides of the invention described herein. The basic parameters affecting the choice of hybridization conditions and guidance for devising suitable conditions are set forth by Sambrook,, Fritsch, and Maniatis (1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., chapters 9 and 11; and Current Protocols in Molecular Biology, 1995, Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4), and can be readily determined by those having ordinary skill in the art based on, for example, the length and/or base composition of the DNA. One way of achieving moderately stringent conditions involves the use of a prewashing solution containing 5 x SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0), hybridization buffer of about 50% formamide, 6 x 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 x SSC, 0.1% SDS. Generally, highly stringent conditions are defined as hybridization conditions as above, but with washing at approximately 68 degrees C, 0.2 x SSC, 0.1% SDS. SSPE (1xSSPE 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 (1xSSC 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 1xSSC = 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.

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 can include contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes can 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 can include 10000 basepairs (more preferably, 5000 basepairs, still 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 coding sequence of the cytokine polypeptide of the invention, and 10000 basepairs (more preferably, 5000 basepairs, still 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 coding sequence of the cytokine polypeptide of the invention. 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 Cytokine Polypeptides of the Invention

Methods for making cytokine polypeptides of the invention 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.

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 United States Patent 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. Patent 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 is one that promotes insertion of the polypeptide into cell membranes, and most preferably, promotes extracellular secretion of the polypeptide from that host cell. The signal peptide is preferably cleaved from the polypeptide upon membrane insertion or 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.

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 is CHO strain DX-B11, 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-B11, 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.

Alternatively, cytokine gene products of the invention 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. Patent 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.

A number of types of cells can 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) (McMahan *et al.*, 1991, *EMBO J.* 10: 2821, 1991), human embryonic kidney cells such as 293, 293 EBNA or MSR 293, human epidermal A431 cells, human Colo205 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HL-60, U937, HaK or Jurkat cells. Optionally, mammalian cell lines such as HepG2/3B, KB, NIH 3T3 or S49, for example, can be used for expression of the polypeptide when it is desirable to use the polypeptide in various signal transduction or reporter assays. Alternatively, it is possible to produce the polypeptide in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Suitable yeasts include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces strains, Candida, or any yeast strain capable of expressing heterologous polypeptides. 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 desirable 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 can be accomplished using known chemical or enzymatic methods. The polypeptide can 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). 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".

The polypeptide of the invention can be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant polypeptide. The resulting expressed polypeptide can then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as selective precipitation with various salts, gel filtration, and ion exchange chromatography. The purification of the polypeptide can 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 using an antibody that specifically binds one or more epitopes of cytokine polypeptides of the invention. Alternatively, the polypeptide of the invention can also be expressed in a form which will facilitate purification. For example, it can be expressed as a fusion polypeptide, that is, it may be fused with maltose binding polypeptide (MBP), glutathione-S-transferase (GST), thioredoxin (TRX), a polyHis peptide, and/or fragments thereof. Kits for expression and purification of such fusion polypeptides are commercially available from New England BioLabs (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 cytokine polypeptides of the invention, fragments, variants, binding partners etc. The polypeptide of the invention can 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.

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.

The polypeptide can 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 cytokine polypeptides of the invention can possess biological properties in common therewith, including cytokine polypeptide activity. Thus, they can 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.

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 Cytokine Polypeptides of the Invention

Any method which neutralizes cytokine polypeptides of the invention or inhibits expression of genes encoding cytokine polypeptides of the invention (either transcription or translation) can be used to reduce the biological activities of cytokine polypeptides of the invention. In particular embodiments, antagonists inhibit the binding to cells of at least one cytokine polypeptide of the invention, thereby inhibiting biological activities induced by the binding of those cytokine polypeptides of the invention to the cells. In certain other embodiments of the invention, antagonists can be designed to reduce the level of endogenous expression for the gene encoding a polypeptide of the invention, *e.g.*, using well-known antisense or ribozyme approaches to inhibit or prevent translation of such cytokine mRNA transcripts; triple helix approaches to inhibit transcription of such cytokine genes; or targeted

homologous recombination to inactivate or "knock out" said cytokine genes or their endogenous promoters or enhancer elements. Such antisense, ribozyme, and triple helix antagonists can be designed to reduce or inhibit either unimpaired, or if appropriate, mutant cytokine gene activity. Peptide agonists and antagonists of activities of the polypeptides of the invention can also be identified and utilized (see, for example, WO 00/24782 and WO 01/83525, which are incorporated by reference herein). Techniques for the production and use of such molecules are well known to those of skill in the art.

Antisense RNA and DNA molecules act to directly block the translation of mRNA by hybridizing to targeted mRNA and preventing polypeptide translation. Antisense approaches involve the design of oligonucleotides (either DNA or RNA) that are complementary to an mRNA corresponding to a cytokine polypeptide of the invention. 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 can thus be tested, or triplex formation can be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Preferred oligonucleotides 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 cytokine gene transcript, or to the coding regions, could be used in an antisense approach to inhibit translation of endogenous mRNA encoding a cytokine polypeptide of the invention. 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 can include other appended groups such as peptides (*e.g.*, for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, Proc Natl Acad Sci U.S.A. 86: 6553-6556; Lemaitre *et al.*, 1987, Proc Natl Acad Sci 84: 648-652; PCT Publication No. WO88/09810), 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 cytokine 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 endogenous cytokine gene transcripts and thereby prevent translation of the cytokine 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.

Ribozyme molecules designed to catalytically cleave cytokine mRNA transcripts can also be used to prevent translation of mRNA and expression of cytokine polypeptides of the invention. (See, *e.g.*, PCT International Publication WO90/11364 and US Patent 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 cytokine 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 sufficient quantities of the ribozyme to destroy endogenous messages encoding cytokine polypeptides of the invention and inhibit translation of such polypeptides. Because ribozymes, unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Alternatively, endogenous gene expression of the cytokines of the invention 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 IMX7189 cytokine 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).

Anti-sense RNA and DNA, ribozyme, and triple helix molecules of the invention can 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 can 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 can be generated by in vitro and in vivo transcription of DNA sequences encoding the antisense RNA molecule. Such DNA sequences can 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.

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, Tabara, and Mello, 2000, Genetic requirements for inheritance of RNAi in C. elegans, Science 287 (5462): 2494-2497), or the introduction of transgenes (Dernburg *et al.*, 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.

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).

Also encompassed within the invention are variants of cytokine polypeptide of the invention with partner binding sites that have been altered in conformation so that (1) the cytokine 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 cytokine 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: 395-401). Nucleic acids encoding such altered cytokine polypeptides of the invention can be introduced into organisms according to methods described herein, and can replace the endogenous nucleic acid sequences encoding the corresponding cytokine polypeptide. Such methods allow for the interaction of a particular cytokine polypeptide of the invention with its binding partners to be regulated by administration of a small molecule compound to an organism, either systemically or in a localized manner.

The cytokine polypeptides of the invention themselves can also be employed in inhibiting a biological activity of cytokines of the invention in *in vitro* or *in vivo* procedures. Encompassed within the invention are mutated regions of cytokine polypeptides of the invention that act as "dominant negative" inhibitors of native cytokine polypeptide function when expressed as fragments or as components of fusion polypeptides. For example, an altered polypeptide region of the present invention can be used to inhibit binding of cytokine polypeptides of the invention to endogenous binding partners. Such use effectively would block cytokine polypeptide interactions and inhibit cytokine polypeptide activities. Furthermore, antibodies which bind to cytokine polypeptides of the invention often inhibit cytokine polypeptide activity and act as antagonists. For example, antibodies that specifically recognize one or more epitopes of cytokine polypeptides of the invention, or epitopes

of conserved variants of cytokine polypeptides of the invention, or peptide fragments of the cytokine polypeptides of the invention can be used in the invention to inhibit cytokine polypeptide 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 cytokine polypeptides of the invention of the present invention can be administered to modulate interactions between cytokine polypeptides of the invention and cytokine binding partners that are not membrane-bound. Such an approach will allow an alternative method for the modification of cytokine-influenced bioactivity.

In an alternative aspect, the invention further encompasses the use of agonists of activity of the cytokine polypeptides of the invention to treat or ameliorate the symptoms of a disease for which increased cytokine polypeptide activity is beneficial. In a preferred aspect, the invention entails administering compositions comprising a cytokine nucleic acid or a cytokine polypeptide of the invention 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 cytokines of the invention are soluble forms, as described above. In still another aspect of the invention, the compositions comprise administering a cytokine-encoding nucleic acid for expression of a cytokine polypeptide of the invention 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 cytokine polypeptide of the invention. Furthermore, the invention encompasses the administration to cells and/or organisms of compounds found to increase the endogenous activity of cytokine polypeptides of the invention. One example of compounds that increase cytokine polypeptide activity are agonistic antibodies, preferably monoclonal antibodies, that bind to cytokine polypeptides of the invention or binding partners, which may increase the activity of cytokine polypeptides of the invention by causing constitutive intracellular signaling (or "ligand mimicking"), or by preventing the binding of a native inhibitor of the activity of a cytokine polypeptide of the invention.

Another approach to development of therapeutic agents is peptide library screening. The interaction of a protein ligand with its receptor often takes place at a relatively large interface. However, as demonstrated for human growth hormone and its receptor, only a few key residues at the interface contribute to most of the binding energy (Clackson *et al.*, 1995, *Science* 267: 383-386). The bulk of the protein ligand merely displays the binding epitopes in the right topology or serves functions unrelated to binding. Thus, molecules of only "peptide" length (2 to 90 amino acids) can bind to the receptor protein or binding partner of even a large protein ligand such as a polypeptide of the invention. Such peptides may mimic the bioactivity of the large protein ligand ("peptide agonists") or, through competitive binding, inhibit the bioactivity of the large protein ligand ("peptide antagonists"). Exemplary peptide agonists and antagonists of polypeptides of the invention may comprise a domain of a naturally occurring molecule or may comprise randomized sequences. The term "randomized" as

used to refer to peptide sequences refers to fully random sequences (e.g., selected by phage display methods or RNA-peptide screening) and sequences in which one or more residues of a naturally occurring molecule is replaced by an amino acid residue not appearing in that position in the naturally occurring molecule. Phage display peptide libraries have emerged as a powerful method in identifying such peptide agonists and antagonists. See, for example, Scott *et al.*, 1990, *Science* 249: 386; Devlin *et al.*, 1990, *Science* 249: 404; U.S. Pat. No. 5,223,409; U.S. Pat. No. 5,733,731; U.S. Pat. No. 5,498,530; U.S. Pat. No. 5,432,018; U.S. Pat. No. 5,338,665; U.S. Pat. No. 5,922,545; WO 96/40987; and WO 98/15833 (each of which is incorporated by reference in its entirety). In such libraries, random peptide sequences are displayed by fusion with coat proteins of filamentous phage. Typically, the displayed peptides are affinity-eluted against an antibody-immobilized extracellular domain of a receptor. The retained phages may be enriched by successive rounds of affinity purification and repropagation. The best binding peptides may be sequenced to identify key residues within one or more structurally related families of peptides. The peptide sequences may also suggest which residues may be safely replaced by alanine scanning or by mutagenesis at the DNA level. Mutagenesis libraries may be created and screened to further optimize the sequence of the best binders (Lowman, 1997, *Ann. Rev. Biophys. Biomol. Struct.* 26: 401-424). Another biological approach to screening soluble peptide mixtures uses yeast for expression and secretion (Smith et al., 1993, *Mol. Pharmacol.* 43: 741-748) to search for peptides with favorable therapeutic properties. Hereinafter, this and related methods are referred to as "yeast-based screening." A peptide library can also be fused to the carboxyl terminus of the lac repressor and expressed in *E. coli*. Another *E. coli*-based method allows display on the cell's outer membrane by fusion with a peptidoglycan-associated lipoprotein (PAL). Hereinafter, these and related methods are collectively referred to as "*E. coli* display." In another method, translation of random RNA is halted prior to ribosome release, resulting in a library of polypeptides with their associated RNA still attached. Hereinafter, this and related methods are collectively referred to as "ribosome display." Other methods employ peptides linked to RNA; for example, PROfusion technology, Phylos, Inc. (*see*, for example, Roberts and Szostak, 1997, *Proc. Natl. Acad. Sci. USA* 94: 12297-12303). Hereinafter, this and related methods are collectively referred to as "RNA-peptide screening." Chemically derived peptide libraries have been developed in which peptides are immobilized on stable, non-biological materials, such as polyethylene rods or solvent-permeable resins. Another chemically derived peptide library uses photolithography to scan peptides immobilized on glass slides. Hereinafter, these and related methods are collectively referred to as "chemical-peptide screening." Chemical-peptide screening may be advantageous in that it allows use of D-amino acids and other unnatural analogues, as well as non-peptide elements. Both biological and chemical methods are reviewed in Wells and Lowman, 1992, *Curr. Opin. Biotechnol.* 3: 355-362.

In the case of known bioactive peptides, rational design of peptide ligands with favorable therapeutic properties can be completed. In such an approach, one makes stepwise changes to a peptide sequence and determines the effect of the substitution upon bioactivity or a predictive biophysical property of the peptide (e.g., solution structure). Hereinafter, these techniques are collectively referred

to as "rational design." In one such technique, one makes a series of peptides in which one replaces a single residue at a time with alanine. This technique is commonly referred to as an "alanine walk" or an "alanine scan." When two residues (contiguous or spaced apart) are replaced, it is referred to as a "double alanine walk." The resultant amino acid substitutions can be used alone or in combination to result in a new peptide entity with favorable therapeutic properties. Structural analysis of protein-protein interaction may also be used to suggest peptides that mimic the binding activity of large protein ligands. In such an analysis, the crystal structure may suggest the identity and relative orientation of critical residues of the large protein ligand, from which a peptide may be designed (*see*, e.g., Takasaki *et al.*, 1997, *Nature Biotech.* 15: 1266-1270). Hereinafter, these and related methods are referred to as "protein structural analysis." These analytical methods may also be used to investigate the interaction between a receptor protein and peptides selected by phage display, which may suggest further modification of the peptides to increase binding affinity.

Peptide agonists and antagonists of polypeptides of the invention may be covalently linked to a vehicle molecule. The term "vehicle" refers to a molecule that prevents degradation and/or increases half-life, reduces toxicity, reduces immunogenicity, or increases biological activity of a therapeutic protein. Exemplary vehicles include an Fc domain or a linear polymer (e.g., polyethylene glycol (PEG), polylysine, dextran, etc.); a branched-chain polymer (*see*, for example, U.S. Pat. No. 4,289,872; U.S. Pat. No. 5,229,490; WO 93/21259); a lipid; a cholesterol group (such as a steroid); a carbohydrate or oligosaccharide (e.g., dextran); or any natural or synthetic protein, polypeptide or peptide that binds to a salvage receptor.

Antibodies to Cytokine Polypeptides of the Invention

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 cytokine polypeptides of the invention, 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 cytokine polypeptides of the invention, fragments, variants, fusion polypeptides, etc., as set forth above can be employed as "immunogens" in producing antibodies immunoreactive therewith.

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.

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, NY, (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 can 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. Other techniques known to those of skill in the art, such as phage display or ribosome display methods, can be used to produce antibodies specific for particular epitopes of cytokine polypeptides of the invention.

For the production of antibodies, various host animals can be immunized by injection with one or more of the following: a cytokine polypeptide of the invention, a fragment of said cytokine polypeptide, a functional equivalent of said cytokine polypeptide, or a mutant form of said cytokine polypeptide. Such host animals can include but are not limited to rabbits, guinea pigs, mice, and rats. Various adjuvants can 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 can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof.

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: 643-646; 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. Patent 6,054,297. Procedures to generate antibodies transgenically can be found in GB 2,272,440, US Patent 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, NJ) and Abgenix Inc. (Fremont, CA). In another preferred embodiment, fully human antibodies for use in humans are produced by screening a phage display library of human antibody variable domains (Vaughan et al., 1998, Nat Biotechnol. 16(6): 535-539; and U.S. Patent No. 5,969,108).

Antigen-binding antibody fragments that recognize specific epitopes can 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 can 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 cytokine 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 can 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 cytokine polypeptide of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" said cytokine polypeptide and that may bind to the cytokine polypeptide's binding partners 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).

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. Patent 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. Patent 4,474,893; and U.S. Patent 6,106,833). U.S. Patent 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. Patent 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. Immnol.* 148:1547-4553; 1992). U.S. Patent 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. Patent 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. Patent 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).

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 cytokine polypeptides of the invention, induce biological effects (e.g., transduction of biological signals) similar to the biological effects induced when the cytokine binding partner binds to cell surface cytokine polypeptide. Agonistic antibodies can be used to induce cytokine-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 cytokine polypeptides of the invention of the invention via a first antigen binding domain will be useful in diagnostic applications and in treating conditions and diseases involving the proliferation or the development of cells from pluripotent stem cell precursors.

Those antibodies that can block binding of the cytokine polypeptides of the invention to binding partners for said cytokines can be used to inhibit cytokine-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 cytokine polypeptides of the invention to certain cells expressing a cytokine binding partner. Alternatively, blocking antibodies can be identified in assays for the ability to inhibit a biological effect that results from binding of soluble cytokine to target cells. Antibodies can be assayed for the ability to inhibit cytokine 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 cytokine polypeptide of the invention 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 cytokine 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 a cytokine polypeptide of the invention, and a physiologically acceptable diluent, excipient, or carrier, are provided herein. Suitable components of such compositions are as described below for compositions containing cytokine polypeptides of the invention.

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 Cytokine Polypeptides of the Invention

The purified cytokine polypeptides of the invention of the invention (including polypeptides, polypeptides, fragments, variants, oligomers, and other forms) are useful in a variety of assays. For example, the cytokines of the present invention can be used to identify binding partners of the cytokine polypeptides of the invention, 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.

Assays to Identify Binding Partners. Cytokine polypeptides of the invention 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 cytokine polypeptide of the invention 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.

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 x 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, MA) 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 RM, 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 cytokine polypeptides of the invention include but are not limited to small organic molecules, such as those that are commercially available - often as part of large combinatorial chemistry compound 'libraries' - from companies such as Sigma-Aldrich (St. Louis, MO), Arqule (Woburn, MA), Enzymed (Iowa City, IA), Maybridge Chemical Co.(Trevillett, Cornwall, UK), MDS Panlabs (Bothell, WA), Pharmacopeia (Princeton, NJ), and Trega (San Diego, CA). Preferred small organic molecules for screening using these assays are usually less than 10K molecular weight and can 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 cytokine polypeptides of the invention.

Yeast Two-Hybrid or "Interaction Trap" Assays. Where the cytokine polypeptide of the invention binds or potentially binds to another polypeptide (such as, for example, in a receptor-ligand interaction), the nucleic acid encoding the cytokine polypeptide of the invention 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.

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 cytokine polypeptide of the invention and intact cells expressing said cytokine (endogenous or recombinant) on the cell surface. For example, a radiolabeled soluble cytokine fragment can be used to compete with a soluble cytokine 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, NJ.

Assays to Identify Modulators of Intercellular Communication, Cell Stimulation, or Immune Cell Activity. The influence of the cytokine polypeptides of the invention on intercellular communication, cell stimulation, or immune cell activity can be manipulated to control these activities in target cells. For example, the disclosed cytokine polypeptides of the invention, nucleic acids encoding the disclosed cytokine polypeptides of the invention, 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 cytokine polypeptides of the invention, 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 a cytokine polypeptide of the invention 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 a cytokine polypeptide of the invention. In such an assay, one would determine a rate of communication or cell stimulation in the presence of said cytokine polypeptide and then determine if such communication or cell stimulation is altered in the presence of a candidate agonist or antagonist or another cytokine 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.

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 cytokine polypeptide of the invention 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 contacted with the cytokine 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 cytokine polypeptide of the invention 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.

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 cytokine polypeptide of the invention may, among other means, be measured by the following methods:

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

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.

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.

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. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988

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.

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.

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.

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.

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.

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

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.

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, NY; 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, NY; 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, NY; 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, NY; 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, NY.

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).

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

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.

Diagnostic and Other Uses of Cytokine Polypeptides and Nucleic Acids of the Invention

The nucleic acids encoding the cytokine polypeptides of the invention 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 cytokine polypeptides of the invention 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

Probes and Primers. Among the uses of the disclosed cytokine nucleic acids, nucleic acids encoding cytokine polypeptides of the invention, 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 cytokine homologues.

Diagnostics and Gene Therapy. The nucleic acids encoding cytokine polypeptides of the invention, 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.

Methods of Screening for Binding Partners. The cytokine polypeptides of the invention of the invention each can be used as reagents in methods to screen for or identify binding partners. For example, the cytokine polypeptides of the invention 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, NJ). 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 cytokine polypeptides of the invention also find use in identifying cells that express a cytokine binding partner on the cell surface. Purified cytokine polypeptides of the invention 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, cytokine polypeptides of the invention 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.

Measuring Biological Activity. Cytokine polypeptides of the invention also find use in measuring the biological activity of cytokine-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.

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, ^{109}Pd, ^{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, Illinois). A number of techniques for radiolabeling polypeptides are known. Radionuclide metals can be attached to polypeptides by using a suitable bifunctional chelating agent. 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 Cytokine Polypeptides of the Invention and Antagonists Thereof

The cytokine polypeptides of the invention, 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 cytokine polypeptides of the invention may have effects similar to or different from cytokine polypeptides of the invention. For example, an antagonist of the stimulation of cell proliferation activity of cytokine polypeptides of the invention 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 cytokine polypeptide of the invention may also act as an effective dominant negative antagonist of that activity. Therefore, in the following paragraphs "cytokine polypeptides of the invention or antagonists" refers to all cytokine polypeptides of the invention, fragments, variants, antagonists, agonists, antibodies, and binding partners etc. of the invention, 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.

Administration of Cytokine Polypeptides of the Invention and Antagonists Thereof

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 disorder mediated by a cytokine polypeptide of the invention. Such cytokine-mediated disorders include conditions caused (directly or indirectly) or exacerbated by binding between a cytokine polypeptide of the invention 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, cytokine polypeptides of the invention and fragments, nucleic acids encoding said cytokine polypeptides, and/or agonists or antagonists (such as antibodies) of cytokine polypeptides of the invention 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 cytokine polypeptides of the invention.

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 cytokine polypeptide of the invnetion. "Therapeutic agent" includes without limitation any of the cytokine polypeptides of the invention, fragments, and variants; nucleic acids encoding the cytokine polypeptides of the invention, fragments, and variants; agonists or antagonists of the cytokine polypeptides of the invention such as antibodies; cytokine polypeptide binding partners; complexes formed from the cytokine polypeptides of the invention, 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 days, or more preferably, by one or more weeks. The degree of improvement is determined based on signs or symptoms, and determinations can also employ questionnaires that are administered to the patient, such as quality-of-life questionnaires. Various indicators that reflect the extent of the patient's illness can be assessed for determining whether the amount and time of the treatment is sufficient. The baseline value for the chosen indicator or indicators is established by examination of the patient prior to administration of the first dose of the therapeutic agent. Preferably, the baseline examination is done within about 60 days of administering the first dose. If the therapeutic agent is being administered to treat acute symptoms, the first dose is administered as soon as practically possible after the injury has occurred. Improvement is induced by administering therapeutic agents such as cytokine polypeptides of the invention 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 injuries or other acute conditions. Although the extent of the patient's illness after treatment may appear improved according to one or more indicators, treatment may be continued indefinitely at the same level or at a reduced dose or frequency. Once treatment has been reduced or discontinued, it later may be resumed at the original level if symptoms should reappear.

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 can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC50 (*i.e.*, the concentration of the test compound which achieves

a half-maximal inhibition of symptoms) as determined in cell culture, while minimizing toxicities. Such information can be used to more accurately determine useful doses in humans. Ultimately, the attending physician will decide the amount of polypeptide of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of polypeptide of the present invention and observe the patient's response. Larger doses of polypeptide of the present invention can be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various 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, cytokine polypeptides of the invention 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 cytokine polypeptides of the invention 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 can be administered, whose amount may range from 5-100 mg/dose. Exemplary dose ranges for a flat dose to be administered by subcutaneous injection are 5-25 mg/dose, 25-50 mg/dose and 50-100 mg/dose. In one embodiment of the invention, the various indications described below are treated by administering a preparation acceptable for injection containing cytokine polypeptides of the invention or antagonists at 25 mg/dose, or alternatively, containing 50 mg per dose. The 25 mg or 50 mg dose can be administered repeatedly, particularly for chronic conditions. If a route of administration other than injection is used, the dose is appropriately adjusted in accord with standard medical practices. In many instances, an improvement in a patient's condition will be obtained by injecting a dose of about 25 mg of cytokine polypeptides of the invention or antagonists one to three times per week over a period of at least three weeks, or a dose of 50 mg of cytokine polypeptides of the invention 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 can be continued indefinitely, with adjustments being made to dose and frequency if such are deemed necessary by the patient's physician. The foregoing doses are examples for an adult patient who is a person who is 18 years of age or older. For pediatric patients (age 4-17), a suitable regimen involves the subcutaneous injection of 0.4 mg/kg, up to a maximum dose of 25 mg of cytokine polypeptides of the invention or antagonists, administered by subcutaneous injection one or more times per week. If an antibody against a cytokine polypeptide of the invention is used as the cytokine 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-cytokine 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 can be injected or administered intravenously.

Formulations. Compositions comprising an effective amount of a cytokine 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 can 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 can 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 cytokine polypeptides of the invention are used. Sustained-release forms suitable for use in the disclosed methods include, but are not limited to, cytokine polypeptides of the invention 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.

Combinations of Therapeutic Compounds. A cytokine 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 cytokine polypeptides of the invention or antagonists concurrently with one or more other drugs that are administered to the same

patient in combination with the cytokine polypeptides of the invention 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 can be administered in the same or in separate compositions, and by the same or different routes of administration. Examples of components that can be administered concurrently with the pharmaceutical compositions of the invention are: cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, 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, IL-23, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin, or inhibitors or antagonists of any of these factors. The pharmaceutical composition can further contain other agents which either enhance the activity of the polypeptide or compliment its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with polypeptide of the invention, or to minimize side effects. Conversely, a cytokine polypeptide or antagonist of the present invention may be included in formulations of the particular cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent. 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, cytokine polypeptides of the invention or antagonists can be combined with a second such cytokine polypeptide/antagonist, including an antibody against a cytokine polypeptide, or a cytokine polypeptide-derived peptide that acts as a competitive inhibitor of a native cytokine polypeptide of the invnetion.

Routes of Administration. Any efficacious route of administration can be used to therapeutically administer cytokine polypeptides of the invention 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, ice creams, or chewing gum; and topical preparations such as lotions, gels, sprays, ointments or other suitable techniques. Alternatively, polypeptideaceous cytokine polypeptides of the invention or antagonists may be administered by implanting cultured cells that express the polypeptide, for example, by implanting cells that express

cytokine polypeptides of the invention or antagonists. Cells may also be cultured ex vivo in the presence of polypeptides of the present invention in order to modulate cell proliferation or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes. The polypeptide of the instant invention may also be administered by the method of protein transduction. In this method, the cytokine polypeptide of the invention is covalently linked to a protein-transduction domain (PTD) such as, but not limited to, TAT, Antp, or VP22 (Schwarze *et al.*, 2000, *Cell Biology* 10: 290-295). The PTD-linked peptides can then be transduced into cells by adding the peptides to tissue-culture media containing the cells (Schwarze *et al.*, 1999, *Science* 285: 1569; Lindgren *et al.*, 2000, *TiPS* 21: 99; Derossi *et al.*, 1998, *Cell Biology* 8: 84; WO 00/34308; WO 99/29721; and WO 99/10376). In another embodiment, the patient's own cells are induced to produce cytokine polypeptides of the invention or antagonists by transfection *in vivo* or *ex vivo* with a DNA that encodes cytokine polypeptides of the invention or antagonists. This DNA can be introduced into the patient's cells, for example, by injecting naked DNA or liposome-encapsulated DNA that encodes cytokine polypeptides of the invention or antagonists, or by other means of transfection. Nucleic acids of the invention can also be administered to patients by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA). When cytokine polypeptides of the invention or antagonists are administered in combination with one or more other biologically active compounds, these can be administered by the same or by different routes, and can be administered simultaneously, separately or sequentially.

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 can 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 can be added. The liquid form of the pharmaceutical composition can 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.

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 can 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.

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 can 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, can 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 can 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 can 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 can 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 can 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 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 polypeptides 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 can 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.

Veterinary Uses. In addition to human patients, cytokine polypeptides of the invention 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 condition mediated by a cytokine polypeptide of the invention. In such instances, an appropriate dose can 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, cytokine polypeptides of the invention 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 can be administered indefinitely.

Manufacture of Medicaments. The present invention also relates to the use of cytokine polypeptides of the invention, fragments, and variants; nucleic acids encoding the cytokine polypeptides of the invention, fragments, and variants; agonists or antagonists of the cytokine

polypeptides of the invention such as antibodies; cytokine polypeptide binding partners; complexes formed from the cytokine polypeptides of the invention , fragments, variants, and binding partners, etc, in the manufacture of a medicament for the prevention or therapeutic treatment of each medical disorder disclosed herein.

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

EXAMPLE 1

Identification of IMX7189 Polypeptides, and Additional Polypeptides With Cytokine Structures

A data set was received from Celera Genomics (Rockville, Maryland) containing a listing of amino acid sequences predicted, using automated approaches such as the GENSCAN program (Miyajima *et al.*, 2000, *Biochem Biophys Res Commun* 272: 801-807) and Otto (Venter *et al.*, 2001, *Science* 291: 1304-1351), to be encoded by the human genome. These amino acid sequence predictions were analyzed using GeneFold (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) (Berman *et al.*, 2000, *Nucleic Acids Res* 28: 235-242). 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 classify 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 Interleukin-4 (IL-4), Interleukin-6 (IL-6), 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, 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 assigning a new protein to membership in a known family of proteins.

When one of the polypeptides predicted from the human genome data (IMX7189) was threaded into the GeneFold program, several of the highest-scoring template structures for this polypeptide were cytokine or growth factor templates, although structural similarities to other alpha-helix containing proteins were also identified. This predicted polypeptide sequence was then used to identify an

assembly of human EST sequences that encode it, and oligonucleotide primers were designed on the basis of the assembled sequences. Using the IMX7189 oligonucleotide primers, PCR reactions were performed on a panel of cDNAs derived from RNA samples from different human tissues. Amplification of a single cDNA band was observed from most tissue samples, with absent or significantly reduced amplification of cDNA from skeletal muscle. The resulting human IMX7189 cDNA molecule (SEQ ID NO:1) encodes an IMX7189 cytokine polypeptide having the amino acid sequence shown in SEQ ID NO:2; nucleotides 203 through 619 of SEQ ID NO:1 encode SEQ ID NO:2, with nucleotides 620 through 622 of SEQ ID NO:1 corresponding to a stop codon. Amino acids sequences similar to that of human IMX7189 polypeptide have been reported in databases; for example the "FLEXHT-49" polypeptide of WO 00/70047 (GeneSeq AAB36627), the "SEQ ID NO 3395" polypeptide of WO 01/53312 (GeneSeq AAM40250); TrEMBL database accession numbers Q9BST1 and Q9NWKO, and GenBank accession numbers AAH04818, XP_040852.1, and BAA91380.1. A variant of human IMX7189 polypeptide with an altered C-terminal sequence has been reported in TrEMBL database accession number Q9P0R6 and GenBank accession number NP_057556 and is discussed further below. Two truncated human IMX7189 amino acid sequences have been reported in WO 00/55171 (GeneSeq AAB28000) and WO 00/61620 (GeneSeq AAB51684). However, none of these disclosures of sequences related to human IMX7189 polypeptide have identified the disclosed polypeptides as 4AHB cytokines or even as having alpha-helical structure. An amino acid sequence related to IMX168745 has subsequently been disclosed in GenBank accession number XM_062633, furthermore the GenBank XM_062633 sequence differs from IMX168745 throughout the alpha helical bundle region of IMX168745, and was not identified in the GenBank database entry as a cytokine or even as having alpha-helical structure. An amino acid sequence related to a portion of IMX185787 has subsequently been disclosed in GenBank accession number XM_062575, furthermore the GenBank XM_062575 sequence is missing the majority of the alpha helical bundle region of IMX185787, and was not identified in the GenBank database entry as a cytokine or even as having alpha-helical structure. A mouse amino acid sequence that apparently represents the murine homolog of IMX188339 has been disclosed as GenBank accession number XM_137866, the disclosed murine amino acid sequence seems to have an extraneous 183-aa N-terminal extension relative to the human sequence, however, thus an alpha-helical polypeptide of the present invention is the amino acid sequence of GenBank XM_137866 from amino acid 184 through 977, and fragments thereof having cytokine polypeptide activity. The alpha helical bundle region within the murine XM_137866 sequence is predicted to begin approximately between amino acids 775 and 776 of this sequence and end approximately between amino acids 912 and 918 of the XM_137866 amino acid sequence. Partial human amino sequences lacking the alpha helical bundle region of IMX188339 have been disclosed in EP 1104808 A1 (and the related publication JP 2002010789) and subsequently in GenBank accession number BAC04083.1.

The human IMX7189 nucleotide sequences were used to identify the corresponding murine homologue through analysis of combined murine EST and genomic sequences. Oligonucleotide

primers designed using the predicted mouse IMX7189 cDNA sequence were used in PCR reactions performed on a panel of cDNAs derived from RNA samples from different murine tissues. Amplification of a single cDNA band was also observed from murine tissue samples. The predicted murine IMX7189 cDNA molecule (SEQ ID NO:3) encodes a murine IMX7189 cytokine polypeptide having the amino acid sequence shown in SEQ ID NO:4; nucleotides 187 through 618 of SEQ ID NO:3 encode SEQ ID NO:4, with nucleotides 619 through 621 of SEQ ID NO:1 corresponding to a stop codon and nucleotides 1726 through 1731 likely representing a polyadenylation signal for the poly(A) tail at nucleotides 1766 (or 1767) through 1782. The cDNA for the murine IMX7189 cytokine polypeptide encodes two potential initiator methionines, one at position 1 of SEQ ID NO:4 and another at position 6 of SEQ ID NO:4. A preferred embodiment of the invention is the amino acid sequence of SEQ ID NO:4 from amino acid 6 through amino acid 144.

The IMX7189 human cytokine coding sequences were compared with publicly available preliminary human genomic DNA sequences, and the following chromosome 14q32.3 contigs were identified as containing IMX7189 cytokine coding sequences: AC015863.3 and AL359240.4. The approximate positions of the exons containing IMX7189 cytokine coding sequence in the AC015863.3 contig are shown in the table below, along with their locations relative to SEQ ID NO:1; note that the 5' and 3' untranslated regions may extend further along the contig sequence beyond those portions that correspond to SEQ ID NO:1, as indicated by the parentheses around the AC015863.3 endpoints in the table.

Correspondence of IMX7189 gene exons in human contig AC015863.3 and in cDNA sequence:

IMX7189 Exons	Position in AC015863.3	Position in SEQ ID NO:1
Exon 1	(119047)-119146	1-100
Exon 2	135266-135366	101-201
Exon 3	137825-138083	202-460
Exon 4	141103-(141270)	461-628

The genomic sequences comprising human IMX7189 cytokine exons map to the 14q32.3 region of human chromosome 14. Human IMX7189 nucleic acids such as SEQ ID NO:1 and fragments thereof are useful for the cytological identification of this chromosomal region, and for the genomic mapping of human heritable disorders such as the following disorders that have been genetically mapped to this region: Usher syndrome, Type IA (USH1A); microphthalmos, autosomal recessive (MCOP); ectopic expression of creatine kinase, brain type (CKBE); Fahr disease (idiopathic basal ganglia calcification; BGCI; IBGC; nonarteriosclerotic cerebral calcification; striopallidodentate calcinosis; SPD calcinosis; cerebrovascular ferrocalcinosis); myopathy, distal 1, late distal hereditary (MPD1); multinodular goiter 1 (MNG1; goiter, nontoxic, with intrathyroidal calcification; adolescent multinodular goiter; euthyroid goiter; simple goiter); hereditary benign chorea (BCH; BHC; hereditary progressive chorea without dementia); and achromatopsia 1 (ACHM1; rod monochromatism 1; rod monochromacy 1; RMCH1).

Additional variations of cytokine polypeptides of the invention are provided, including naturally occurring genomic variants of the IMX7189 cytokine sequences disclosed herein. As one

example, amino acid 109 of human IMX7189 (SEQ ID NO:2) differs from amino acid 109 of a naturally occurring variant of human IMX7189, where the change from a Ser residue to a Pro residue was apparently caused by a single change from 'T' at position 527 of SEQ ID NO:1 to 'C'. This variation and others are listed in the table below, including inter-species amino acid differences between human and murine cytokine polypeptides of the invention. Such variations may be incorporated into an IMX7189 cytokine polypeptide or nucleic acid individually or in any combination, or in combination with alternative splice variations. An additional alteration in the human IMX7189 coding sequence has been reported (see for example GenBank Accession No. NM_016472); this sequence shows an additional 'A' residue inserted between nucleotides 593 and 596 of SEQ ID NO:1, causing a frameshift that produces the amino acid sequence reported at GenBank Accession No. NP_057556 and shown in SEQ ID NO:5. If this altered human IMX7189 coding sequence represents a naturally occurring genomic variant, and not a sequencing error, the polypeptide of SEQ ID NO:5 would be considered a human IMX7189 polypeptide.

Allelic Variant Amino Acid Change (human IMX7189)	Position in SEQ ID NO:2	Nucleotide Change	Position in SEQ ID NO:1
none (in 5' UTR)	n/a	A -> G	168
Ser -> Pro	109	T -> C	527
Inter-Species Amino Acid Change (human -> mouse IMX7189)	Position in SEQ ID NO:2 (human IMX7189)		Position in SEQ ID NO:4 (mouse IMX7189)
Cys -> Tyr	5		10
Met -> Val	8		13
Thr -> Ala	28		33
Arg -> Gln	34		39
Asn -> His	50		55
Leu -> Met	57		62
Lys -> Arg	86		91
Asp -> Glu	96		101

The amino acid sequences of human and murine cytokine polypeptides of the invention (SEQ ID NOs 2 and 4) were compared with each other and with related polypeptides using the GCG "pretty" multiple sequence alignment program, with amino acid similarity scoring matrix = blosum62, gap creation penalty = 8, and gap extension penalty = 2. An alignment of these sequences is shown in Table 1, and includes consensus residues which are identical among four of the amino acid sequences in the alignment. The capitalized residues in the alignment are those which match the consensus residues. Amino acid substitutions and other alterations (deletions, insertions, etc.) to IMX7189 cytokine amino acid sequences (e.g. SEQ ID NOs 2 and 4) are predicted to be more likely to alter or disrupt IMX7189 cytokine polypeptide activities if they result in changes to the capitalized residues of the amino acid sequences as shown in Table, and particularly if those changes do not substitute an amino acid of similar chemical properties (such as substitution of any one of the aliphatic residues -

Ala, Gly, Leu, Ile, or Val - for another aliphatic residue), or a residue present in other cytokine polypeptides at that conserved position. Conversely, if a change is made to an IMX7189 cytokine amino acid sequence resulting in substitution of the residue at that position in the alignment from one of the other Table 1 cytokine polypeptide sequences, it is less likely that such an alteration will affect the function of the altered IMX7189 cytokine polypeptide. For example, the consensus residue at position 58 in Table 1 is glutamate (Glu), but one of the IMX7189-related polypeptides has a leucine (Leu) at that position; substitution of the chemically similar aspartate (Asp), or of leucine or another of the aliphatic amino acids, for glutamate at that position is less likely to alter the function of the polypeptide than substitution of tryptophan or tyrosine etc. Embodiments of the invention include cytokine polypeptides of the invention and fragments of cytokine polypeptides of the invention, comprising altered amino acid sequences. Altered IMX7189 cytokine polypeptide sequences share at least 30%, or more preferably at least 40%, or more preferably at least 50%, or more preferably at least 55%, or more preferably at least 60%, or more preferably at least 65%, or more preferably at least 70%, or more preferably at least 75%, or more preferably at least 80%, or more preferably at least 85%, or more preferably at least 90%, or more preferably at least 95%, or more preferably at least 97.5%, or more preferably at least 99%, or most preferably at least 99.5% amino acid identity with one or more of the cytokine amino acid sequences shown in Table 1. When IMX7189 cytokine polypeptide variants according to the invention, such as allelic variants or cytokine polypeptides of the invention having deliberately engineered modifications, are analyzed using GeneFold as described further herein, at least one of the ten top-scoring template structures within one of the three types of GeneFold scoring methods will be cytokine or growth factor polypeptides. The score for the top-scoring cytokine or growth factor template structures, using any of the three types of score reported by GeneFold (sequence only, sequence plus local conformation preferences plus burial terms, or sequence plus local conformation preferences plus burial terms plus secondary structure) preferably will be at least 20, more preferably at least 30, more preferably at least 40, still more preferably at least 50, and most preferably at least 60.

Table 1: Amino acid sequence alignment of IMX7189 cytokines with related polypeptides

	SEQ ID	1 50
hIMX7189	NO:2	~~~~~~~~~~ ~~~~~~~~~~m etDcnpmElS Sm...sGfee GseLnGfegt
hIMX7189v	NO:5	~~~~~~~~~~ ~~~~~~~~~~m etDcnpmElS Sm...sGfee GseLnGfegt
mIMX7189	NO:4	~~~~~~~~~~ ~~~~mgarrm etDynpvElS Sm...sGfee GseLnGfega
ceQ22757	NO:10	~~~~~~~~~~ ~~~~~mtqqs dkDasvsEtt tppttpvtvk kpaLcGscdc
ceQ9XWX6	NO:11	mlksepipcl rcgnvgsptg svpmslhkvS SinrsaGt.. npasrGg...
dmQ9V8F3	NO:12	~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~malvqs
dmQ9VNV2	NO:13	~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~~ ~~~~~~~~~m Gepkatadpg
consensus		---------- ---------- --D----E-S S-----G--- G--L-G----
		51 100
hIMX7189	NO:2	DmkDmrLEaE Av..VNDVlF AVNnmfVSks LrcadDVAYI NVeTkErnrY
hIMX7189v	NO:5	DmkDmrLEaE Av..VNDVlF AVNnmfVSks LrcadDVAYI NVeTkErnrY
mIMX7189	NO:4	DmkDmqLEaE Av..VNDVlF AVNhmfVSks mPcadDVAYI NVeTkErnrY
ceQ22757	NO:10	DvekstLEeE AmaaVrenaF AVNligVSem LPrtsqllfI NVtTfEnhth
ceQ9XWX6	NO:11	...essLElE AiaaVhelsF AVqsisVSem LPrtpDlifv NVtTlEaqpY

dmQ9V8F3	NO:12	DenDfhalrE AqrmVdscqd fadhliVaef LPhcrgVAYI NirTlEqviY
dmQ9VNV2	NO:13	eeqafncEdE AnaiiNDVka hVaeiciSsk LasdatqiYl NirTiEsatc
consensus		D--D--LE-E A---VNDV-F AVN---VS-- LP---DVAYI NV-T-E---Y
		101 150
hIMX7189	NO:2	CLELTeAGlk VVgYaFD.qV dD....hL.. .....q TpYhETvYsL
hIMX7189v	NO:5	CLELTeAGlk VVgYaFD.qV dD....hL.. .....q TpYhETvYsL
mIMX7189	NO:4	CLELTeAGlR VVgYaFD.qV eD....hL.. .....q TpYhETvYsL
ceQ22757	NO:10	CiELTqkGwR VaSnrnDcmn gDfrqldi.. .....h TkYfEslhtL
ceQ9XWX6	NO:11	CLELTlkGwR itSlrsDcmV gDftrleL.. .....f TkYydslYlL
dmQ9V8F3	NO:12	CvqLsrAGyR iVSYeFDdva devancd... ..... TvY.EsahqL
dmQ9VNV2	NO:13	CvqvssrGfk iVSsqyDtid eDsrisaLlr ngqeqgddee eeifETpYaL
consensus		CLELT-AG-R VVSY-FD--V -D-----L-- ---------- T-Y-ET-Y-L
		151 200
hIMX7189	NO:2	LDtlSPaYRE aFG...naLL QRLEalkrdg qs~~~~~~~~ ~~~~~~~~~~
hIMX7189v	NO:5	LDtlSPaYRE aFG...naLL QRLEsfekrw tvmttlfpfr gagagtec~~
mIMX7189	NO:4	LDtlSPaYRE aFG...naLL QRLEalkrdg qs~~~~~~~~ ~~~~~~~~~~
ceQ22757	NO:10	LmdISPlfRE tFG...skLi skLselkker sdsde~~~~~ ~~~~~~~~~~
ceQ9XWX6	NO:11	mDdISPgYRE rFs...ekLv QRLkli..ea geedqvapca slqspslstd
dmQ9V8F3	NO:12	LagISPlYgE kyGfgrepLg kRkEkqs~~~ ~~~~~~~~~~ ~~~~~~~~~~
dmQ9VNV2	NO:13	LDkISPrYvE sFGnqlcqqL raLqqmrtef needeeeeee aeeeekk~~~
consensus		LD-ISP-YRE -FG-----LL QRLE------ ---------- ----------

Table 2 shows a set of amino acid sequences that were identified as having structures similar to known 4AHB cytokines by using the GeneFold programs described above, in combination with other analytical methods. On the basis of this analysis, polypeptides comprising the amino acid sequences of SEQ ID NOs 6 through 9 shown in Table 2 are considered to be potential members of the human 4AHB cytokine family.

IMX168745 polypeptide (SEQ ID NO:6) has a signal sequence from amino acid 1 to approximately amino acid 25 of SEQ ID NO:6, with amino acid 26 of SEQ ID NO:8 being the N-terminal amino acid of the mature polypeptide. The region of IMX168745 polypeptide starting at approximately amino acid 75 through approximately amino acid 255 of SEQ ID NO:6 shows a high-scoring match in GeneFold to the structure of IL-6. Amino acids 28 through 266 of SEQ ID NO:6 also show a significant degree of similarity to putative coding regions of the Mus musculus genome.

The region of IMX185787 polypeptide starting at approximately amino acid 40 through approximately amino acid 240 of SEQ ID NO:7 shows high-scoring matches in GeneFold to the structures of IL-3 and IL-4. Amino acids 97 through 266 of SEQ ID NO:7 also show a significant degree of similarity to putative coding regions of the Mus musculus genome.

The region of IMX188339 polypeptide starting at approximately amino acid 585 through approximately amino acid 735 of SEQ ID NO:8 shows high-scoring matches in GeneFold to the structure of LIF At least some of the predicted exons encoding IMX188339 polypeptide are significantly similar to putative coding regions of the Mus musculus genome. There are several potential initiator methionine residues within SEQ ID NO:8, thus preferred embodiments of the invention are the amino acid sequences of amino acid 1 through amino acid 753 of SEQ ID NO:8,

amino acid 38 through amino acid 753 of SEQ ID NO:8, amino acid 71 through amino acid 753 of SEQ ID NO:8, amino acid 114 through amino acid 753 of SEQ ID NO:8, amino acid 145 through amino acid 753 of SEQ ID NO:8, amino acid 216 through amino acid 753 of SEQ ID NO:8, amino acid 313 through amino acid 753 of SEQ ID NO:8, amino acid 332 through amino acid 753 of SEQ ID NO:8, amino acid 504 through amino acid 753 of SEQ ID NO:8, amino acid 505 through amino acid 753 of SEQ ID NO:8, amino acid 558 through amino acid 753 of SEQ ID NO:8, amino acid 559 through amino acid 753 of SEQ ID NO:8, and amino acid 569 through amino acid 753 of SEQ ID NO:8. Amino acid 754 of SEQ ID NO:8 was determined to be a valine residue in certain sequencing experiments, this may represent an allelic variation between histidine and valine at that position. PCR amplification of IMX188339 cDNA from tissue-specific cDNA panels was performed using oligonucleotide probes based on the IMX188339 coding sequence: IMX188339 cDNA was consistently amplified from cDNA libraries made from adult pancreas and from adult testis, and from fetal brain, indicating expression of IMX188339 mRNA in these tissues.

The region starting at approximately amino acid 25 through approximately amino acid 180 of IMX192967 polypeptide (SEQ ID NO:9) shows high-scoring matches in GeneFold to the structures of IL-4, Interferon tau, and IL-6.

TABLE 2

Identifier:	Amino Acid Sequence:
IMX168745 SEQ ID NO:6	MNCLRSCGCS VLVCLLAGAL LGSTGMHWHL LGLTELGSKL RVRFQSAVSP PSGAAEKASL KLKHTNPFPR RGVALGAKPL RSENSQAAAA FNSSEEGECE QMRQELECVN TPEPAGCFGA GGSEHSTHSN PLGSVPRGRE RAGYPTEKGY HTERCVQGLL KLGLDTGIGI HPLYSITKAS HKVSPDLKGE EEALPLDGSS CKVNIVKYVE TGKGSEEKRN VREEQAVAAG REWVEQSLQL FREKKGALAQ AQEVSLGADV GRNPRISTCG TERSAWQIQS YSDSNVTA
IMX185787 SEQ ID NO:7	MSQTKPDLVK INRRKAKSDC FKMRKIFQDI IMKNIVVLTF SPASGSSTRR KAQLLSDGVK PLDWERRTLQ QFCGVGTANQ LCRTLGPCVS APGGKGEEDV TKVYNTKGRV TMSQELTKEQ KVFYKMVQQL LKAIQCTVES EALHKLMLLI WQECPWLHDQ GTLDLKLREQ LAIQVWKRIP VGQSQGSFVM VRQDATETYI EFINQLQAAI KRQAKIFHYV DDILIAAQYQ SLLHQLYTMM IQEKQKRQVD HYEEDTLGNG RINGRI
IMX188339 SEQ ID NO:8	MNTSRFADHH DLLTETKRPI DTVISQQAFY SDESVSAMEK QYLRNSNLTP QQKIDELHHG FTGLDLEEQW MYPSRSDHSN CHNIQTNDTA KTTFQEYPLI KNCFTPQTGL SDIMKESGVD IYHYGRDRIC TKGLEAPLQQ KRAEMFLSQF NRYNENVDYC RYPEYVHPNK AKLNKCSNFS VQDSKKLANG TPETPTVEAD TYTKLFQVKP ANQKKMEETI PDQQNFTFPK TTPHLTEKQF AKEAVFTADF GLTSEYGLKP HTACPANDFA NVTEKQQFAK PDPPHSEYFK SVNLLSNSAT SSGGIDLNRP TWMNVQTKNN TPIPYRNQGN LMKLNSHLSA ASKGSNHSSD FPQLSSTNLT PNSNLFQKYC QENPSAFSSF DFSYSGAERI QSVNHIEGLT KPGEENLFKL VTDKKIKQPN GFCDNYSAQK YGIIENVNKH NFQAKPQSGH YDPEEGPKHL DGLSQNTYQD LLESQGHSNS HRTRGGDNSR VNRTQVSCFS NNYMMGDLRH NQCFQQLGSN GFPLRSTHPF GHSVVPLLDS YDLLSYDDLS HLYPYFNMMY GDNSFSGLMP TFGFQRPIKT RSGPASELHI RLEECCEQWR ALEKERKKTE LALAKNYPGK KVSSTNNTPV PRLTSNPSRV DRLIVDELRE LARVVTLLGK MERLRSSLLH ASISTALDRH LESIHIVQSR RKDEIVNASN RQRQGVPRCQ DDRDVFALAS AIKEMCVATR KTRTALWCAL QMTLPKTAST ADVHKPLQDT VNCEDKVHES INSSNPMNQR GETNKH
IMX192967 SEQ ID NO:9	GVVRSAVQLF PGTTGSCPLW LSSHRSQATV REALASVARL AANGGVGWSG RPSSNKSGTT GLEADRRHEV CYCAAAAALK LCEAIDLTSM NHNTIDLVSD HCESRNLNSE QQEVKYWLMR MFVCLVSLLI SVLGAGACLA ALESWTISEE LQFFWKKGHW SSMAILASAR RQQTQINRLS PKSSAPICKL LDPKTGCFPS WKVIVFFSFE KRDSYEFGSS RGLVLWEVPF LKGGDKAWRK FGHSLFKDAG LVKMGRHEVI NNFLSLFFST VPHISCVSWK RQASGPW

EXAMPLE 2

Analysis of IMX7189 Cytokine Expression by Real-Time Quantitative PCR

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, TX; Clontech Laboratories, Palo Alto, CA; and Stratagene, La Jolla, CA). The RNA samples were DNase treated (part # 1906, Ambion, Austin, TX), and reverse transcribed into a population of cDNA molecules using TaqMan Reverse Transcription Reagents (part # N808-0234, Applied Biosystems, Foster City, CA) 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.

Sets of probes and oligonucleotide primers complementary to mRNAs encoding human IMX7189 (SEQ ID NO:2) polypeptides were designed using Primer Express software (Applied Biosystems, Foster City, CA) 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. The forward IMX7189 primer used was 5' AGA GCT GAA CGG TTT TGA AGG A 3' (SEQ ID NO:14); the reverse IMX7189 primer used was 5' CAA AGA GAA CAT CAT TTA CAA CTG CTT 3' (SEQ ID NO:15); and the labeled probe used for human IMX7189 was 5' AGC TTC GAG CCT CAT GTC TTT CAT GTC AG 3' (SEQ ID NO:16). 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 both human IMX7189 and GAPDH probe/primer sets were set up in 25-microliter volumes with TAQMAN Universal PCR Master Mix (part # 4304437, Applied Biosystems, Foster City, CA) 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, CA), 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 IMX7189 to GAPDH.

Expression of human IMX7189 relative to GAPDH expression, was analyzed in a variety of adult and fetal RNA samples. This analysis confirmed that human IMX7189 messages are detectable (although less abundant than housekeeper mRNAs) in the adult and fetal tissues tested, with the IMX7189 message levels relative to GAPDH expression generally between 0.1% and 4% of GAPDH levels. Adult skeletal muscle had a lower relative level of expression, approximately 0.02% of GAPDH, consistent with the result described in Example 1 above seen when PCR was used to amplify human IMX7189 RNAs from skeletal muscle. Certain tissues exhibited significant levels of IMX7189

message levels relative to GAPDH expression, such as colon and pancreas (10% and 6% relative to GAPDH, respectively), while the highest levels of human IMX7189 expression were observed in thymus and thyroid tissue (50% and 73% relative to GAPDH, respectively). In addition, expression of human IMX7189 message appeared to be somewhat higher in naive (CD4+ CD25- CD45ROlow) T cells, 3.7% relative to GAPDH, than in memory (CD4+ CD25- CD45ROhigh) or regulatory (CD4+ CD25+) T cells (2.0% and 1.8% relative to GAPDH, respectively).

Analysis of human IMX7189 expression relative to housekeeper gene expression in additional RNA samples indicated that in some cell types, there was a detectable increase in expression of human IMX7189 in cells treated with interferon gamma (IFNg). In the human epithelial colon carcinoma cell line T84, for example, treatment with cytokines such as a mixture of IL-4 and IL-13, or a mixture of IL-1, IL-18, and TNF alpha did not strongly alter relative levels of human IMX7189 expression, but treatment with IFNg increased human IMX7189 expression from 1.6% to 5.5% of GAPDH expression levels. Similarly in the human epithelial lung adenocarcinoma cell line Calu3, in unstimulated cells human IMX7189 expression was 1.1% of GAPDH; treatment with IL-4/IL-13 or with IL-1/IL-18/TNF increased IMX7189 expression to 1.2% or 1.9% of GAPDH, while treatment with IFNg increased human IMX7189 expression to 2.7% of that of GAPDH.

EXAMPLE 3

Monoclonal Antibodies That Bind Polypeptides of the Invention

This example illustrates a method for preparing monoclonal antibodies that bind cytokine polypeptides of the invention. Other conventional techniques may be used, such as those described in U.S. Patent 4,411,993. Suitable immunogens that may be employed in generating such antibodies include, but are not limited to, purified cytokine polypeptide of the invention, an immunogenic fragment thereof, and cells expressing high levels of said cytokine polypeptide or an immunogenic fragment thereof. DNA encoding a cytokine polypeptide of the invention can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3: 165, 1995.

Rodents (BALB/c mice or Lewis rats, for example) are immunized with cytokine 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, MT)), 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.

Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision to test for cytokine polypeptide-specific antibodies by dot-blot assay, ELISA (enzyme-linked immunosorbent assay), immunoprecipitation, or other suitable assays, such as FACS analysis of inhibition of binding of cytokine polypeptide of the invention to a cytokine polypeptide binding partner. Following detection

of an appropriate antibody titer, positive animals are provided one last intravenous injection of cytokine polypeptide of the invention 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.

The hybridoma cells may be screened by ELISA for reactivity against purified cytokine polypeptide of the invention by adaptations of the techniques disclosed in Engvall et al., (*Immunochem. 8*: 871, 1971) and in U.S. Patent 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 the cytokine polypeptide of the invention.

EXAMPLE 4

Antisense Inhibition of Expression of Nucleic Acids Encoding Cytokines of the Invention

In accordance with the present invention, a series of oligonucleotides are designed to target different regions of mRNA molecules encoding cytokine polypeptides of the invention, using the nucleotide sequences of SEQ ID NOs 1 and 3 and nucleic acids encoding SEQ ID Nos 6 through 9 as the bases for the design of the oligonucleotides. Oligonucleotide sequences, such as pools of degenerate oligonucleotides, may be selected that will hybridize to mRNA molecules encoding all of the cytokine polypeptides of the invention, or to mRNA molecules encoding a subset thereof. 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. Methods such as those of Gray and Clark (U.S. Patent Nos 5,856,103 and 6,183,966) can be used to select oligonucleotides that form the most stable hybrid structures with target sequences, as such oligonucleotides are desirable for use as antisense inhibitors.

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. Patent No. 5,948,680. Modified oligonucleosides may also be used in oligonucleotide synthesis, for example methylenemethylimino-linked oligonucleosides,

methylene-dimethylhydrazo-linked oligonucleosides, methylene-carbonylamino-linked oligonucleosides, and methylene-aminocarbonyl-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.

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". Different types of chimeric oligonucleotides may be prepared according to U.S. Patent 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. Oligonucleotides are preferably synthesized via solid phase P(III) phosphoramidite chemistry on an automated synthesizer 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.

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 oligonucleotides 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.

Antisense modulation of cytokine nucleic acid expression can be assayed in a variety of ways known in the art. For example, cytokine 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. Levels of cytokine polypeptides of the invention 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 polypeptides of the invention 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).

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.

Sequences Presented in the Sequence Listing

SEQ ID NO	Type	Description
SEQ ID NO:1	Nucleotide	Human IMX7189 cDNA sequence
SEQ ID NO:2	Amino acid	Human IMX7189 amino acid sequence
SEQ ID NO:3	Nucleotide	Mus musculus IMX7189 predicted cDNA sequence
SEQ ID NO:4	Amino acid	Mus musculus IMX7189 predicted amino acid sequence
SEQ ID NO:5	Amino acid	Variant of human IMX7189 amino acid sequence (GenBank Accession No. NP_057556)
SEQ ID NO:6	Amino acid	Human IMX168745 amino acid sequence
SEQ ID NO:7	Amino acid	Human IMX185787 amino acid sequence
SEQ ID NO:8	Amino acid	Human IMX188339 amino acid sequence
SEQ ID NO:9	Amino acid	Human IMX192967 amino acid sequence
SEQ ID NO:10	Amino acid	Caenorhabditis elegans amino acid sequence (TrEMBL Q22757)
SEQ ID NO:11	Amino acid	Caenorhabditis elegans amino acid sequence (TrEMBL Q9XWX6)
SEQ ID NO:12	Amino acid	Drosophila melanogaster amino acid sequence (TrEMBL Q9V8F3)
SEQ ID NO:13	Amino acid	Drosophila melanogaster amino acid sequence (TrEMBL Q9VNV2)

SEQUENCE LISTING

<110> Immunex Corporation
Baum, Peter R.
Ketchem, Randal R.
Mosley, Bruce A.

<120> HUMAN AND MURINE CYTOKINE POLYPEPTIDES

<130> 3361-WO

<160> 16

<170> PatentIn version 3.1

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<212> DNA
<213> Homo sapiens

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gtcaggattt gaagaaggtt cagagctgaa cggttttgaa ggaactgaca tgaaagacat 300

gaggctcgaa gctgaagcag ttgtaaatga tgttctcttt gctgttaaca acatgtttgt 360

ctcgaaaagc ctgcggtgtg cggatgatgt ggcctatatc aatgtggaaa caaaggaaag 420

aaacagatat tgcctagaac tcactgaagc agggctcaag gtggtaggct atgcttttga 480

ccaggtagat gatcatttac agactcccta ccatgaaaca gtctactcct tgttggatac 540

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<210> 2
<211> 139
<212> PRT
<213> Homo sapiens

<400> 2
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Glu Glu Gly Ser Glu Leu Asn Gly Phe Glu Gly Thr Asp Met Lys Asp
20 25 30

Met Arg Leu Glu Ala Glu Ala Val Val Asn Asp Val Leu Phe Ala Val
35 40 45

Asn Asn Met Phe Val Ser Lys Ser Leu Arg Cys Ala Asp Asp Val Ala
50 55 60

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

Thr Glu Ala Gly Leu Lys Val Val Gly Tyr Ala Phe Asp Gln Val Asp
85 90 95

Asp His Leu Gln Thr Pro Tyr His Glu Thr Val Tyr Ser Leu Leu Asp
100 105 110

Thr Leu Ser Pro Ala Tyr Arg Glu Ala Phe Gly Asn Ala Leu Leu Gln
115 120 125

Arg Leu Glu Ala Leu Lys Arg Asp Gly Gln Ser
130 135

<210> 3
<211> 1782
<212> DNA
<213> Mus musculus

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gggcgggggt ggtgccggcg ccgcgatcct ggccccagca gtctccggac gaggaaggat 180

aagaacatgg gcgctcgcag aatggaaaca gactataatc ccgtggagct aagcagtatg 240

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cagctggagg ccgaggcagt tgtaaacgat gtactctttg ctgtcaacca catgtttgtc 360

tcaaaaagca tgccctgtgc agatgatgtg gcttacatca atgtggagac aaaggaaagg 420

aacagatact gcctggagct cacagaagca gggctcaggg tggtgggcta tgcttttgac 480

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cagtgataca gaaattagaa tcacctaact tcagacctgg attcagcctg ctaaatcagg 1020

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cagcagttgg acagtgctct gtctcattgc cacatgaaca gtgtaacatg agtgcattgt 1620

atggttcgaa accaaaggat gaatgaagca ttcagaaact tgatatttaa aaaagagatg 1680

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<211> 144
<212> PRT
<213> Mus musculus

<400> 4

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Ser Met Ser Gly Phe Glu Glu Gly Ser Glu Leu Asn Gly Phe Glu Gly
20 25 30

Ala Asp Met Lys Asp Met Gln Leu Glu Ala Glu Ala Val Val Asn Asp
35 40 45

Val Leu Phe Ala Val Asn His Met Phe Val Ser Lys Ser Met Pro Cys
50 55 60

Ala Asp Asp Val Ala Tyr Ile Asn Val Glu Thr Lys Glu Arg Asn Arg
65 70 75 80

Tyr Cys Leu Glu Leu Thr Glu Ala Gly Leu Arg Val Val Gly Tyr Ala
85 90 95

Phe Asp Gln Val Glu Asp His Leu Gln Thr Pro Tyr His Glu Thr Val
100 105 110

Tyr Ser Leu Leu Asp Thr Leu Ser Pro Ala Tyr Arg Glu Ala Phe Gly
115 120 125

Asn Ala Leu Leu Gln Arg Leu Glu Ala Leu Lys Arg Asp Gly Gln Ser
130 135 140

<210> 5
<211> 155
<212> PRT
<213> Homo sapiens

<400> 5

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Glu Glu Gly Ser Glu Leu Asn Gly Phe Glu Gly Thr Asp Met Lys Asp
20 25 30

Met Arg Leu Glu Ala Glu Ala Val Val Asn Asp Val Leu Phe Ala Val
35 40 45

Asn Asn Met Phe Val Ser Lys Ser Leu Arg Cys Ala Asp Asp Val Ala
50 55 60

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

Thr Glu Ala Gly Leu Lys Val Val Gly Tyr Ala Phe Asp Gln Val Asp
85 90 95

Asp His Leu Gln Thr Pro Tyr His Glu Thr Val Tyr Ser Leu Leu Asp
100 105 110

Thr Leu Ser Pro Ala Tyr Arg Glu Ala Phe Gly Asn Ala Leu Leu Gln
115 120 125

Arg Leu Glu Ser Phe Glu Lys Arg Trp Thr Val Met Thr Thr Leu Phe
130 135 140

Pro Phe Arg Gly Ala Gly Ala Gly Thr Glu Cys
145 150 155

<210> 6
<211> 288
<212> PRT
<213> Homo sapiens

<400> 6

Met Asn Cys Leu Arg Ser Cys Gly Cys Ser Val Leu Val Cys Leu Leu
1 5 10 15

Ala Gly Ala Leu Leu Gly Ser Thr Gly Met His Trp His Leu Leu Gly
20 25 30

Leu Thr Glu Leu Gly Ser Lys Leu Arg Val Arg Phe Gln Ser Ala Val
35 40 45

Ser Pro Pro Ser Gly Ala Ala Glu Lys Ala Ser Leu Lys Leu Lys His
50 55 60

Thr Asn Pro Phe Pro Arg Arg Gly Val Ala Leu Gly Ala Lys Pro Leu
65 70 75 80

Arg Ser Glu Asn Ser Gln Ala Ala Ala Ala Phe Asn Ser Ser Glu Glu
85 90 95

Gly Glu Cys Glu Gln Met Arg Gln Glu Leu Glu Cys Val Asn Thr Pro
100 105 110

Glu Pro Ala Gly Cys Phe Gly Ala Gly Gly Ser Glu His Ser Thr His
115 120 125

Ser Asn Pro Leu Gly Ser Val Pro Arg Gly Arg Glu Arg Ala Gly Tyr
130 135 140

Pro Thr Glu Lys Gly Tyr His Thr Glu Arg Cys Val Gln Gly Leu Leu
145 150 155 160

Lys Leu Gly Leu Asp Thr Gly Ile Gly Ile His Pro Leu Tyr Ser Ile
165 170 175

Thr Lys Ala Ser His Lys Val Ser Pro Asp Leu Lys Gly Glu Glu Glu
180 185 190

Ala Leu Pro Leu Asp Gly Ser Ser Cys Lys Val Asn Ile Val Lys Tyr
195 200 205

Val Glu Thr Gly Lys Gly Ser Glu Glu Lys Arg Asn Val Arg Glu Glu
210 215 220

Gln Ala Val Ala Ala Gly Arg Glu Trp Val Glu Gln Ser Leu Gln Leu
225 230 235 240

Phe Arg Glu Lys Lys Gly Ala Leu Ala Gln Ala Gln Glu Val Ser Leu
245 250 255

Gly Ala Asp Val Gly Arg Asn Pro Arg Ile Ser Thr Cys Gly Thr Glu
260 265 270

Arg Ser Ala Trp Gln Ile Gln Ser Tyr Ser Asp Ser Asn Val Thr Ala
275 280 285

<210> 7
<211> 266
<212> PRT
<213> Homo sapiens

<400> 7

Met Ser Gln Thr Lys Pro Asp Leu Val Lys Ile Asn Arg Arg Lys Ala
1 5 10 15

Lys Ser Asp Cys Phe Lys Met Arg Lys Ile Phe Gln Asp Ile Ile Met
20 25 30

Lys Asn Ile Val Val Leu Thr Phe Ser Pro Ala Ser Gly Ser Ser Thr
35 40 45

Arg Arg Lys Ala Gln Leu Leu Ser Asp Gly Val Lys Pro Leu Asp Trp
50 55 60

Glu Arg Arg Thr Leu Gln Gln Phe Cys Gly Val Gly Thr Ala Asn Gln
65 70 75 80

Leu Cys Arg Thr Leu Gly Pro Cys Val Ser Ala Pro Gly Gly Lys Gly
85 90 95

Glu Glu Asp Val Thr Lys Val Tyr Asn Thr Lys Gly Arg Val Thr Met
100 105 110

Ser Gln Glu Leu Thr Lys Glu Gln Lys Val Phe Tyr Lys Met Val Gln
115 120 125

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

Lys Leu Met Leu Leu Ile Trp Gln Glu Cys Pro Trp Leu His Asp Gln
145 150 155 160

Gly Thr Leu Asp Leu Lys Leu Arg Glu Gln Leu Ala Ile Gln Val Trp
165 170 175

Lys Arg Ile Pro Val Gly Gln Ser Gln Gly Ser Phe Val Met Val Arg
180 185 190

Gln Asp Ala Thr Glu Thr Tyr Ile Glu Phe Ile Asn Gln Leu Gln Ala
195 200 205

Ala Ile Lys Arg Gln Ala Lys Ile Phe His Tyr Val Asp Asp Ile Leu
210 215 220

Ile Ala Ala Gln Tyr Gln Ser Leu Leu His Gln Leu Tyr Thr Met Met
225 230 235 240

Ile Gln Glu Lys Gln Lys Arg Gln Val Asp His Tyr Glu Glu Asp Thr
245 250 255

Leu Gly Asn Gly Arg Ile Asn Gly Arg Ile
260 265

<210> 8
<211> 786
<212> PRT
<213> Homo sapiens

<400> 8

Met Asn Thr Ser Arg Phe Ala Asp His His Asp Leu Leu Thr Glu Thr
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Lys Arg Pro Ile Asp Thr Val Ile Ser Gln Gln Ala Phe Tyr Ser Asp
20 25 30

Glu Ser Val Ser Ala Met Glu Lys Gln Tyr Leu Arg Asn Ser Asn Leu
35 40 45

Thr Pro Gln Gln Lys Ile Asp Glu Leu His His Gly Phe Thr Gly Leu
50 55 60

Asp Leu Glu Glu Gln Trp Met Tyr Pro Ser Arg Ser Asp His Ser Asn
65 70 75 80

Cys His Asn Ile Gln Thr Asn Asp Thr Ala Lys Thr Thr Phe Gln Glu
85 90 95

Tyr Pro Leu Ile Lys Asn Cys Phe Thr Pro Gln Thr Gly Leu Ser Asp
100 105 110

Ile Met Lys Glu Ser Gly Val Asp Ile Tyr His Tyr Gly Arg Asp Arg
115 120 125

Ile Cys Thr Lys Gly Leu Glu Ala Pro Leu Gln Gln Lys Arg Ala Glu
130 135 140

Met Phe Leu Ser Gln Phe Asn Arg Tyr Asn Glu Asn Val Asp Tyr Cys
145 150 155 160

Arg Tyr Pro Glu Tyr Val His Pro Asn Lys Ala Lys Leu Asn Lys Cys
165 170 175

Ser Asn Phe Ser Val Gln Asp Ser Lys Lys Leu Ala Asn Gly Thr Pro
180 185 190

Glu Thr Pro Thr Val Glu Ala Asp Thr Tyr Thr Lys Leu Phe Gln Val
195 200 205

Lys Pro Ala Asn Gln Lys Lys Met Glu Glu Thr Ile Pro Asp Gln Gln
210 215 220

Asn Phe Thr Phe Pro Lys Thr Thr Pro His Leu Thr Glu Lys Gln Phe
225 230 235 240

Ala Lys Glu Ala Val Phe Thr Ala Asp Phe Gly Leu Thr Ser Glu Tyr
245 250 255

Gly Leu Lys Pro His Thr Ala Cys Pro Ala Asn Asp Phe Ala Asn Val
260 265 270

Thr Glu Lys Gln Gln Phe Ala Lys Pro Asp Pro Pro His Ser Glu Tyr
275 280 285

Phe Lys Ser Val Asn Leu Leu Ser Asn Ser Ala Thr Ser Ser Gly Gly
290 295 300

Ile Asp Leu Asn Arg Pro Thr Trp Met Asn Val Gln Thr Lys Asn Asn
305 310 315 320

Thr Pro Ile Pro Tyr Arg Asn Gln Gly Asn Leu Met Lys Leu Asn Ser
325 330 335

His Leu Ser Ala Ala Ser Lys Gly Ser Asn His Ser Ser Asp Phe Pro
340 345 350

Gln Leu Ser Ser Thr Asn Leu Thr Pro Asn Ser Asn Leu Phe Gln Lys
355 360 365

Tyr Cys Gln Glu Asn Pro Ser Ala Phe Ser Ser Phe Asp Phe Ser Tyr
370 375 380

Ser Gly Ala Glu Arg Ile Gln Ser Val Asn His Ile Glu Gly Leu Thr
385 390 395 400

Lys Pro Gly Glu Glu Asn Leu Phe Lys Leu Val Thr Asp Lys Lys Ile
405 410 415

Lys Gln Pro Asn Gly Phe Cys Asp Asn Tyr Ser Ala Gln Lys Tyr Gly
420 425 430

Ile Ile Glu Asn Val Asn Lys His Asn Phe Gln Ala Lys Pro Gln Ser
435 440 445

Gly His Tyr Asp Pro Glu Glu Gly Pro Lys His Leu Asp Gly Leu Ser
450 455 460

Gln Asn Thr Tyr Gln Asp Leu Leu Glu Ser Gln Gly His Ser Asn Ser
465 470 475 480

His Arg Thr Arg Gly Gly Asp Asn Ser Arg Val Asn Arg Thr Gln Val
485 490 495

Ser Cys Phe Ser Asn Asn Tyr Met Met Gly Asp Leu Arg His Asn Gln
500 505 510

Cys Phe Gln Gln Leu Gly Ser Asn Gly Phe Pro Leu Arg Ser Thr His
515 520 525

Pro Phe Gly His Ser Val Val Pro Leu Leu Asp Ser Tyr Asp Leu Leu
530 535 540

Ser Tyr Asp Asp Leu Ser His Leu Tyr Pro Tyr Phe Asn Met Met Tyr
545 550 555 560

Gly Asp Asn Ser Phe Ser Gly Leu Met Pro Thr Phe Gly Phe Gln Arg
565 570 575

Pro Ile Lys Thr Arg Ser Gly Pro Ala Ser Glu Leu His Ile Arg Leu
580 585 590

Glu Glu Cys Cys Glu Gln Trp Arg Ala Leu Glu Lys Glu Arg Lys Lys
595 600 605

Thr Glu Leu Ala Leu Ala Lys Asn Tyr Pro Gly Lys Lys Val Ser Ser
610 615 620

Thr Asn Asn Thr Pro Val Pro Arg Leu Thr Ser Asn Pro Ser Arg Val
625 630 635 640

Asp Arg Leu Ile Val Asp Glu Leu Arg Glu Leu Ala Arg Val Val Thr
645 650 655

Leu Leu Gly Lys Met Glu Arg Leu Arg Ser Ser Leu Leu His Ala Ser
660 665 670

Ile Ser Thr Ala Leu Asp Arg His Leu Glu Ser Ile His Ile Val Gln
675 680 685

Ser Arg Arg Lys Asp Glu Ile Val Asn Ala Ser Asn Arg Gln Arg Gln
690 695 700

Gly Val Pro Arg Cys Gln Asp Asp Arg Asp Val Phe Ala Leu Ala Ser
705 710 715 720

Ala Ile Lys Glu Met Cys Val Ala Thr Arg Lys Thr Arg Thr Ala Leu
725 730 735

Trp Cys Ala Leu Gln Met Thr Leu Pro Lys Thr Ala Ser Thr Ala Asp
740 745 750

Val His Lys Pro Leu Gln Asp Thr Val Asn Cys Glu Asp Lys Val His
755 760 765

Glu Ser Ile Asn Ser Ser Asn Pro Met Asn Gln Arg Gly Glu Thr Asn
770 775 780

Lys His
785

<210> 9
<211> 287
<212> PRT
<213> Homo sapiens

<400> 9

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

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

Ala Leu Ala Ser Val Ala Arg Leu Ala Ala Asn Gly Gly Val Gly Trp
35 40 45

Ser Gly Arg Pro Ser Ser Asn Lys Ser Gly Thr Thr Gly Leu Glu Ala
50 55 60

Asp Arg Arg His Glu Val Cys Tyr Cys Ala Ala Ala Ala Ala Leu Lys
65 70 75 80

Leu Cys Glu Ala Ile Asp Leu Thr Ser Met Asn His Asn Thr Ile Asp
85 90 95

Leu Val Ser Asp His Cys Glu Ser Arg Asn Leu Asn Ser Glu Gln Gln
100 105 110

Glu Val Lys Tyr Trp Leu Met Arg Met Phe Val Cys Leu Val Ser Leu
115 120 125

Leu Ile Ser Val Leu Gly Ala Gly Ala Cys Leu Ala Ala Leu Glu Ser
130 135 140

Trp Thr Ile Ser Glu Glu Leu Gln Phe Phe Trp Lys Lys Gly His Trp
145 150 155 160

Ser Ser Met Ala Ile Leu Ala Ser Ala Arg Arg Gln Gln Thr Gln Ile
165 170 175

Asn Arg Leu Ser Pro Lys Ser Ser Ala Pro Ile Cys Lys Leu Leu Asp
180 185 190

Pro Lys Thr Gly Cys Phe Pro Ser Trp Lys Val Ile Val Phe Phe Ser
195 200 205

Phe Glu Lys Arg Asp Ser Tyr Glu Phe Gly Ser Ser Arg Gly Leu Val
210 215 220

Leu Trp Glu Val Pro Phe Leu Lys Gly Gly Asp Lys Ala Trp Arg Lys
225 230 235 240

Phe Gly His Ser Leu Phe Lys Asp Ala Gly Leu Val Lys Met Gly Arg
245 250 255

His Glu Val Ile Asn Asn Phe Leu Ser Leu Phe Phe Ser Thr Val Pro
260 265 270

His Ile Ser Cys Val Ser Trp Lys Arg Gln Ala Ser Gly Pro Trp
275 280 285

<210> 10
<211> 156
<212> PRT
<213> Caenorhabditis elegans

<400> 10

Met Thr Gln Gln Ser Asp Lys Asp Ala Ser Val Ser Glu Thr Thr Thr
1 5 10 15

Pro Pro Thr Thr Pro Val Thr Val Lys Lys Pro Ala Leu Cys Gly Ser
20 25 30

Cys Asp Cys Asp Val Glu Lys Ser Thr Leu Glu Glu Glu Ala Met Ala
35 40 45

Ala Val Arg Glu Asn Ala Phe Ala Val Asn Leu Ile Gly Val Ser Glu
50 55 60

Met Leu Pro Arg Thr Ser Gln Leu Leu Phe Ile Asn Val Thr Thr Phe
65 70 75 80

Glu Asn His Thr His Cys Ile Glu Leu Thr Gln Lys Gly Trp Arg Val
85 90 95

Ala Ser Asn Arg Asn Asp Cys Met Asn Gly Asp Phe Arg Gln Leu Asp
100 105 110

Ile His Thr Lys Tyr Phe Glu Ser Leu His Thr Leu Leu Met Asp Ile
115 120 125

Ser Pro Leu Phe Arg Glu Thr Phe Gly Ser Lys Leu Ile Ser Lys Leu
130 135 140

Ser Glu Leu Lys Lys Glu Arg Ser Asp Ser Asp Glu
145 150 155

<210> 11

<211> 210
<212> PRT
<213> Caenorhabditis elegans

<400> 11

Met Leu Lys Ser Glu Pro Ile Pro Cys Leu Arg Cys Gly Asn Val Gly
1 5 10 15

Ser Pro Thr Gly Ser Val Pro Met Ser Leu His Lys Val Ser Ser Ile
20 25 30

Asn Arg Ser Ala Gly Thr Asn Pro Ala Ser Arg Gly Gly Glu Ser Ser
35 40 45

Leu Glu Leu Glu Ala Ile Ala Ala Val His Glu Leu Ser Phe Ala Val
50 55 60

Gln Ser Ile Ser Val Ser Glu Met Leu Pro Arg Thr Pro Asp Leu Ile
65 70 75 80

Phe Val Asn Val Thr Thr Leu Glu Ala Gln Pro Tyr Cys Leu Glu Leu
85 90 95

Thr Leu Lys Gly Trp Arg Ile Thr Ser Leu Arg Ser Asp Cys Met Val
100 105 110

Gly Asp Phe Thr Arg Leu Glu Leu Phe Thr Lys Tyr Tyr Asp Ser Leu
115 120 125

Tyr Leu Leu Met Asp Asp Ile Ser Pro Gly Tyr Arg Glu Arg Phe Ser
130 135 140

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

Gln Val Ala Pro Cys Ala Ser Leu Gln Ser Pro Ser Leu Ser Thr Asp
165 170 175

Ser Ser Ser Lys Glu Ser Gln Tyr Ser Ser Thr Glu Ser Leu Pro Ile
180 185 190

Val Thr Pro Val Ser Thr Pro Ala Ile Asp Pro Asp Phe Lys Pro Lys
195 200 205

Phe Lys
210

<210> 12
<211> 119
<212> PRT
<213> Drosophila melanogaster

<400> 12

Met Ala Leu Val Gln Ser Asp Glu Asn Asp Phe His Ala Leu Arg Glu
1 5 10 15

Ala Gln Arg Met Val Asp Ser Cys Gln Asp Phe Ala Asp His Leu Ile
20 25 30

Val Ala Glu Phe Leu Pro His Cys Arg Gly Val Ala Tyr Ile Asn Ile
35 40 45

Arg Thr Leu Glu Gln Val Ile Tyr Cys Val Gln Leu Ser Arg Ala Gly
50 55 60

Tyr Arg Ile Val Ser Tyr Glu Phe Asp Asp Val Ala Asp Glu Val Ala
65 70 75 80

Asn Cys Asp Thr Val Tyr Glu Ser Ala His Gln Leu Leu Ala Gly Ile
85 90 95

Ser Pro Leu Tyr Gly Glu Lys Tyr Gly Phe Gly Arg Glu Pro Leu Gly
100 105 110

Lys Arg Lys Glu Lys Gln Ser
115

<210> 13
<211> 158
<212> PRT
<213> Drosophila melanogaster

<400> 13

Met Gly Glu Pro Lys Ala Thr Ala Asp Pro Gly Glu Glu Gln Ala Phe
1 5 10 15

Asn Cys Glu Asp Glu Ala Asn Ala Ile Ile Asn Asp Val Lys Ala His
20 25 30

Val Ala Glu Ile Cys Ile Ser Ser Lys Leu Ala Ser Asp Ala Thr Gln
35 40 45

Ile Tyr Leu Asn Ile Arg Thr Ile Glu Ser Ala Thr Cys Cys Val Gln
50 55 60

Val Ser Ser Arg Gly Phe Lys Ile Val Ser Ser Gln Tyr Asp Thr Ile
65 70 75 80

Asp Glu Asp Ser Arg Ile Ser Ala Leu Leu Arg Asn Gly Gln Glu Gln
85 90 95

Gly Asp Asp Glu Glu Glu Glu Ile Phe Glu Thr Pro Tyr Ala Leu Leu
100 105 110

Asp Lys Ile Ser Pro Arg Tyr Val Glu Ser Phe Gly Asn Gln Leu Cys
115 120 125

Gln Gln Leu Arg Ala Leu Gln Gln Met Arg Thr Glu Phe Asn Glu Glu
130 135 140

Asp Glu Glu Glu Glu Glu Glu Ala Glu Glu Glu Glu Lys Lys
145 150 155

<210> 14
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide primer

<400> 14
agagctgaac ggttttgaag ga 22

<210> 15
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide primer

<400> 15
caaagagaac atcatttaca actgctt 27

<210> 16
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> oligonucleotide probe

<400> 16
agcttcgagc ctcatgtctt tcatgtcag 29

CLAIMS

What is claimed is:

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

(a) an amino acid sequence that begins between amino acid A through B and ends between amino acid Y through Z, wherein sets of values for A, B, Y, and Z are selected from the group consisting of: A = 35, B = 40, Y = 52, and Z = 53 of SEQ ID NO:2 or of SEQ ID NO:5; A = 72, B = 74, Y = 95, and Z = 98 of SEQ ID NO:2 or of SEQ ID NO:5; A = 98, B = 101, Y = 122, and Z = 123 of SEQ ID NO:2 or of SEQ ID NO:5; and A = 123, B = 124, Y = 136, and Z = 139 of SEQ NO:2 or of SEQ ID NO:5;

(b) a fragment of an amino acid sequence of any of (a) comprising at least 20 contiguous amino acids;

(c) a fragment of an amino acid sequence of any of (a) comprising at least 30 contiguous amino acids;

(d) a fragment of an amino acid sequence of any of (a) having cytokine polypeptide activity;

(e) a fragment of an amino acid sequence of any of (a) comprising Helix A, Helix B. Helix C, and/ or Helix D amino acid sequences;

(f) amino acid sequences comprising at least 20 amino acids and sharing amino acid identity with the amino acid sequences of any of (a)-(e), 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%;

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

(h) an amino acid sequence of (f) or (g) having cytokine polypeptide activity;

wherein said isolated polypeptide does not comprise the amino acid sequence of any of the polypeptides disclosed in WO 00/70047 (GeneSeq AAB36627), WO 01/53312 (GeneSeq AAM40250), TrEMBL database accession numbers Q9BST1 and Q9NWKO, GenBank accession numbers XP_040852.1 and BAA91380.1, TrEMBL database accession number Q9P0R6, GenBank accession number NP_057556, WO 00/55171 (GeneSeq AAB28000), and WO 00/61620 (GeneSeq AAB51684).

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

(a) the amino acid sequence of SEQ ID NO:4;

(b) an amino acid sequence that begins between amino acid A through B and ends between amino acid Y through Z, wherein sets of values for A, B, Y, and Z are selected from the group consisting of: A = 40, B = 45, Y = 57, and Z = 58 of SEQ ID NO:4; A = 77, B = 79, Y = 100, and Z = 103 of SEQ ID NO:4; A = 103, B = 106, Y = 127, and Z = 128 of SEQ ID NO:4; and A = 128, B = 129, Y = 141, and Z = 144 of SEQ NO:4;

(c) a fragment of an amino acid sequence of any of (a)-(b) comprising at least 20 contiguous amino acids;

(d) a fragment of an amino acid sequence of any of (a)-(b) comprising at least 30 contiguous amino acids;

(e) a fragment of an amino acid sequence of any of (a)-(b) having cytokine polypeptide activity;

(f) a fragment of an amino acid sequence of any of (a)-(b) comprising Helix A and/ or Helix D amino acid sequences;

(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%;

(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

(i) an amino acid sequence of (g) or (h) having cytokine polypeptide activity;

wherein said isolated polypeptide does not comprise the amino acid sequence of any of the polypeptides disclosed in WO 00/70047 (GeneSeq AAB36627), WO 01/53312 (GeneSeq AAM40250), TrEMBL database accession numbers Q9BST1 and Q9NWKO, GenBank accession numbers XP_040852.1 and BAA91380.1, TrEMBL database accession number Q9P0R6, GenBank accession number NP_057556, WO 00/55171 (GeneSeq AAB28000), and WO 00/61620 (GeneSeq AAB51684).

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

(a) an amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9;

(b) an amino acid sequence that begins between amino acid A through B and ends between amino acid Y through Z, wherein sets of values for A, B, Y, and Z are selected from the group consisting of: A = 73, B = 94, Y = 108, and Z = 118 of SEQ ID NO:6; A = 153, B = 153, Y = 161, and Z = 173 of SEQ ID NO:6; A = 190, B = 191, Y = 212, and Z = 212 of SEQ NO:6; A = 213, B = 218, Y = 253, and Z = 254 of SEQ ID NO:6; A = 44, B = 47, Y = 82, and Z = 83 of SEQ ID NO:7; A = 114, B = 120, Y = 132, and Z = 152 of SEQ NO:7; A = 140, B = 165, Y = 176, and Z = 178 of SEQ ID NO:7; A = 195, B = 198, Y = 240, and Z = 243 of SEQ ID NO:7; A = 587, B =

588, Y = 613, and Z = 615 of SEQ NO:8; A = 639, B = 643, Y = 664, and Z = 669 of SEQ ID NO:8; A = 673, B = 674, Y = 700, and Z = 702 of SEQ ID NO:8; A = 715, B = 715, Y = 724, and Z = 730 of SEQ NO:8; A = 27, B = 29, Y = 39, and Z = 41 of SEQ NO:9; A = 66, B = 68, Y = 80, and Z = 101 of SEQ ID NO:9; A = 111, B = 111, Y = 133, and Z = 143 of SEQ ID NO:9; and A = 147, B = 148, Y = 177, and Z = 187 of SEQ NO:9;

(c) a fragment of an amino acid sequence of any of (a)-(b) comprising at least 20 contiguous amino acids;

(d) a fragment of an amino acid sequence of any of (a)-(b) comprising at least 30 contiguous amino acids;

(e) a fragment of an amino acid sequence of any of (a)-(b) having cytokine polypeptide activity;

(f) a fragment of an amino acid sequence of any of (a)-(b) comprising Helix A and/ or Helix D amino acid sequences;

(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%;

(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

(i) an amino acid sequence of (g) or (h) having cytokine polypeptide activity.

4. The polypeptide of any of claims 1 through 3, wherein the polypeptide has cytokine polypeptide activity.

5. An isolated nucleic acid encoding a polypeptide of any of claims 1 through 4.

6. The nucleic acid of claim 5 consisting essentially of a nucleotide sequence selected from the group consisting of:

(a) nucleotides 203 through 619 of SEQ ID NO:1;

(b) nucleotides 187 through 618 of SEQ ID NO:3; and

(c) allelic variants of (a)-(b).

7. The nucleic acid of claim 5 consisting essentially of a nucleotide sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:3.

8. An isolated genomic nucleic acid corresponding to the nucleic acid of any of claims 5 through 7.

9. An isolated nucleic acid comprising a nucleotide sequence that shares nucleotide sequence identity with the nucleotide sequences of the nucleic acids of any of claims 5 through 8, 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%, wherein said isolated nucleic acid encodes a polypeptide having cytokine polypeptide activity.

10. An isolated nucleic acid having a length of at least 15 nucleotides, that hybridizes under conditions of moderate stringency to the nucleic acid of any of claims 5 through 9, wherein said isolated nucleic acid encodes a polypeptide having cytokine polypeptide activity.

11. An expression vector comprising at least one nucleic acid according to any of claims 5 though 10.

12. A recombinant host cell comprising at least one nucleic acid according to any of claims 5 through 10.

13. The recombinant host cell of claim 12, wherein the nucleic acid is integrated into the host cell genome.

14. A process for producing a polypeptide encoded by the nucleic acid of any of claims 5 through 10, comprising culturing a recombinant host cell under conditions promoting expression of said polypeptide, wherein the recombinant host cell comprises at least one nucleic acid according to any of claims 5 through 10.

15. The process of claim 14 further comprising purifying said polypeptide.

16. The polypeptide produced by the process of claim 14.

17. An isolated antibody that binds to the polypeptide of any of claims 1 through 4 or claim 16.

18. The antibody of claim 17 wherein the antibody is a monoclonal antibody.

19. The antibody of claim 17 wherein the antibody is a human antibody.

20. The antibody of claim 17 wherein the antibody is a humanized antibody.

21. An isolated antibody wherein the antibody inhibits the activity of the polypeptide of any of claims 1 through 4 or claim 16.

22. A method of designing an inhibitor of the polypeptide of any of claims 1 through 4 or claim 16, the method comprising the steps of determining the three-dimensional structure of 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.

23. A method for identifying compounds that alter cytokine polypeptide activity comprising

(a) mixing a test compound with the polypeptide of any of claims 1 through 4 or claim 16; and

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

24. A method for identifying compounds that inhibit the binding activity of cytokine polypeptides of the invention comprising

(a) mixing a test compound with the polypeptide of any of claims 1 through 4 or claim 16 and a binding partner of said polypeptide; and

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