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(71) Demandeur/Applicant:
HUMAN GENOME SCIENCES, INC., US
(72) Inventeurs/Inventors:
ROSCHKE, VICTOR, US;
ROSEN, CRAIG A., US;
RUBEN, STEVEN M., US
(74) Agent: MBM & CO.

(54) Titre : RECEPTEUR HUMAIN (CCR5) HDGNR10 DE CHIMIOKINE DE LA PROTEINE G
(54) Title: HUMAN G-PROTEIN CHEMOKINE RECEPTOR (CCR5) HDGNR10

(57) **Abrégé/Abstract:**

The present invention relates to a novel human protein called Human G-protein Chemokine Receptor (CCR5) HDGNR10, and isolated polynucleotides encoding this protein. The invention is also directed to human antibodies that bind Human G-protein Chemokine Receptor (CCR5) HDGNR10 and to polynucleotides encoding those antibodies. Also provided are vectors, host cells, antibodies, and recombinant methods for producing Human G-protein Chemokine Receptor (CCR5) HDGNR10 and human anti-Human G-protein Chemokine Receptor (CCR5) HDGNR10 antibodies. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to this novel human protein and these novel human antibodies.



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(71) Applicant (for all designated States except US): **HUMAN GENOME SCIENCES, INC.** [US/US]; 14200 Shady Grove Road, Rockville, MD 20850 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ROSCHKE, Victor** [RU/US]; 13844 Lambertina Place, Rockville, MD 20850 (US). **ROSEN, Craig, A.** [US/US]; 22400 Rolling Hill Lane, Laytonsville, MD 20882 (US). **RUBEN, Steven, M.** [US/US]; 19420 Pyrite Lane, Brookeville, MD 20833 (US).

(74) Agents: **PYTEL, Melissa, J.** et al.; 14200 Shady Grove Road, Rockville, MD 20850 (US).

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(54) Title: HUMAN G-PROTEIN CHEMOKINE RECEPTOR (CCR5) HDGNR10

(57) Abstract: The present invention relates to a novel human protein called Human G-protein Chemokine Receptor (CCR5) HDGNR10, and isolated polynucleotides encoding this protein. The invention is also directed to human antibodies that bind Human G-protein Chemokine Receptor (CCR5) HDGNR10 and to polynucleotides encoding those antibodies. Also provided are vectors, host cells, antibodies, and recombinant methods for producing Human G-protein Chemokine Receptor (CCR5) HDGNR10 and human anti-Human G-protein Chemokine Receptor (CCR5) HDGNR10 antibodies. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating diseases, disorders, and/or conditions related to this novel human protein and these novel human antibodies.



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JUMBO APPLICATIONS / PATENTS

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Human G-protein Chemokine Receptor (CCR5) HDGNR10

Field of the Invention

The present invention relates to a novel human gene encoding a polypeptide which is a member of the G-protein Chemokine Receptor (CCR5) family. More specifically, the present invention relates to a polynucleotide encoding a novel human polypeptide named Human G-protein Chemokine Receptor (CCR5) HDGNR10, referred to herein as "G-protein Chemokine Receptor" or "HDGNR10." This invention also relates to G-protein Chemokine Receptor (CCR5) polypeptides, as well as vectors, host cells, antibodies directed to G-protein Chemokine Receptor (CCR5) polypeptides, and the recombinant methods for producing the same. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the immune system and HIV infection, and therapeutic methods for treating, preventing, and/or diagnosing such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying agonists and antagonists of G-protein Chemokine Receptor (CCR5) activity. The G-protein Chemokine Receptor (CCR5) is also known as CCR5.

Background of the Invention

It is well established that many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second messengers, e.g., cAMP (Lefkowitz, *Nature*, 351:353-354 (1991)). Herein these proteins are referred to as proteins participating in pathways with G-proteins or PPG-proteins. Some examples of these proteins include the GPC receptors, such as those for adrenergic agents and dopamine (Kobilka, B.K., *et al.*, *PNAS*, 84:46-50 (1987); Kobilka, B.K., *et al.*, *Science*, 238:650-656 (1987) ; Bunzow, J.R., *et al.*, *Nature*, 336:783-787 (1988)), G-proteins themselves, effector proteins, e.g., phospholipase C, adenylyl cyclase, and phosphodiesterase, and actuator proteins, e.g., protein kinase A and protein kinase C (Simon, M.I., *et al.*, *Science*, 252:802-8 (1991)).

For example, in one form of signal transduction, the effect of hormone binding is activation of an enzyme, adenylyl cyclase, inside the cell. Enzyme activation by

hormones is dependent on the presence of the nucleotide GTP, and GTP also influences hormone binding. A G-protein connects the hormone receptors to adenylate cyclase. G-protein was shown to exchange GTP for bound GDP when activated by hormone receptors. The GTP-carrying form then binds to an activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself, returns the G-protein to its basal, inactive form. Thus, the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector, and as a clock that controls the duration of the signal.

The membrane protein gene superfamily of G-protein coupled receptors has been characterized as having seven putative transmembrane domains. The domains are believed to represent transmembrane α -helices connected by extracellular or cytoplasmic loops. G-protein coupled receptors include a wide range of biologically active receptors, such as hormone, viral, growth factor and neuroreceptors.

G-protein coupled receptors have been characterized as including these seven conserved hydrophobic stretches of about 20 to 30 amino acids, connecting at least eight divergent hydrophilic loops. The G-protein family of coupled receptors includes dopamine receptors which bind to neuroleptic drugs used for treating psychotic and neurological disorders. Other examples of members of this family include calcitonin, adrenergic, endothelin, cAMP, adenosine, muscarinic, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsins, endothelial differentiation gene-1 receptor and rhodopsins, odorant, cytomegalovirus receptors, etc.

G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (see, Johnson *et al.*, *Endoc., Rev.*, 10:317-331 (1989)). Different G-protein α -subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors have been identified as an important mechanism for the regulation of G-protein coupling of some G-protein coupled receptors. G-protein coupled receptors are found in numerous sites within a mammalian host.

Chemokines, also referred to as intercrine cytokines, are a subfamily of structurally and functionally related cytokines. These molecules are 8-10 kd in size. In general, chemokines exhibit 20% to 75% homology at the amino acid level and are characterized by four conserved cysteine residues that form two disulfide bonds. Based on the arrangement of the first two cysteine residues, chemokines have been classified into two

subfamilies, alpha and beta. In the alpha subfamily, the first two cysteines are separated by one amino acid and hence are referred to as the "C-X-C" subfamily. In the beta subfamily, the two cysteines are in an adjacent position and are, therefore, referred to as the "C-C" subfamily. Thus far, at least nine different members of this family have been identified in humans.

The intercrine cytokines exhibit a wide variety of functions. A hallmark feature is their ability to elicit chemotactic migration of distinct cell types, including monocytes, neutrophils, T lymphocytes, basophils and fibroblasts. Many chemokines have proinflammatory activity and are involved in multiple steps during an inflammatory reaction. These activities include stimulation of histamine release, lysosomal enzyme and leukotriene release, increased adherence of target immune cells to endothelial cells, enhanced binding of complement proteins, induced expression of granulocyte adhesion molecules and complement receptors, and respiratory burst. In addition to their involvement in inflammation, certain chemokines have been shown to exhibit other activities. For example, macrophage inflammatory protein 1 (MIP-1) is able to suppress hematopoietic stem cell proliferation, platelet factor-4 (PF-4) is a potent inhibitor of endothelial cell growth, Interleukin-8 (IL-8) promotes proliferation of keratinocytes, and GRO is an autocrine growth factor for melanoma cells.

In light of the diverse biological activities, it is not surprising that chemokines have been implicated in a number of physiological and disease conditions, including lymphocyte trafficking, wound healing, hematopoietic regulation and immunological disorders such as allergy, asthma and arthritis.

Thus, there is a need for polypeptides that modulate immune system regulation, since disturbances of such regulation may be involved in diseases, disorders, and/or conditions relating to the immune system. Therefore, there is a need for identification and characterization of such human polypeptides which can play a role in detecting, preventing, ameliorating or correcting such diseases, disorders, and/or conditions.

The G-protein Chemokine Receptor (CCR5) is a seven-pass transmembrane G-protein coupled receptor that is expressed in cells of the immune system such as, for example, macrophages, including immature dendritic cells such as Langerhans cells, and T cells, including Th0 and Th1 effector cells. G-protein Chemokine Receptor (CCR5) has also been detected in microglia, astrocytes, neurons, and vascular endothelial cells of the central nervous system (CNS). G-protein Chemokine Receptor (CCR5) is also expressed

in monocytes and T cells in the synovial fluid of rheumatoid arthritis patients, and has also been implicated in other forms of arthritis.

Ligands of G-protein Chemokine Receptor (CCR5) include MIP-1 α , MIP-1 β , MCP-1, MCP-2, MCP-3, MCP-4, RANTES, and Eotaxin. CCR5 is also a major co-receptor for HIV, and may be also be recognized by other infectious agents, such as other viruses, to allow entry into the cell. It was recently discovered that certain individuals harboring a mutation of the CCR5 gene, were resistant to HIV infection despite multiple exposure to the virus. This mutation abrogated expression of CCR5 at the cell surface (Liu et al., Cell 86:1 (1996)).

HIV is currently the leading lethal infectious disease in the world, causing 2.6 million deaths in 1999. The number of deaths resulting from HIV infection will continue to increase; In 1999, there were 5.6 million new cases of HIV infection and 33.6 million infected people living in the world. Although there are currently 14 approved drugs to treat HIV, as many as one half of patients fail to be successfully (with success being defined as no detectable HIV RNA in serum (which in effect is equal to fewer than 50 copies/ml of HIV-1 RNA) treated after a one year drug regimen. The reasons for the inability of these drug regimens to effectively treat HIV are several fold: use of certain drugs results in the development of drug resistant HIV strains; some individuals are intolerant to certain drugs or the drugs have bad side effects; patients have difficulty complying with complex dosing regimens; and the drugs may not be able to access reservoirs of HIV in the body. Thus, there remains a need in the art to develop improved HIV vaccines and therapies.

Summary of the Invention

The present invention relates to novel polynucleotides and the encoded polypeptides of G-protein Chemokine Receptor (CCR5). Moreover, the present invention relates to vectors, host cells, antibodies, and recombinant and synthetic methods for producing the polypeptides and polynucleotides. Also provided are diagnostic methods for detecting diseases, disorders, and/or conditions related to the polypeptides and polynucleotides, and therapeutic methods for treating, preventing, and/or diagnosing such diseases, disorders, and/or conditions. The invention further relates to screening methods for identifying binding partners of G-protein Chemokine Receptor (CCR5).

In accordance with one aspect of the present invention, there are provided novel mature receptor polypeptides as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof. The G-protein Chemokine Receptor (CCR5) polypeptides of the present invention are of human origin.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules encoding the G-protein Chemokine Receptor (CCR5) polypeptides of the present invention, including mRNAs, DNAs, cDNAs, genomic DNA as well as antisense analogs thereof and biologically active and diagnostically or therapeutically useful fragments thereof.

In accordance with a further aspect of the present invention, there are provided processes for producing the G-protein Chemokine Receptor (CCR5) polypeptides by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing nucleic acid sequences encoding the receptor polypeptides of the present invention, under conditions promoting expression of said polypeptides and subsequent recovery of said polypeptides.

In accordance with yet a further aspect of the present invention, there are provided antibodies that bind the G-protein Chemokine Receptor (CCR5) polypeptides. The present invention encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) polypeptide or polypeptide fragment or variant of a G-protein Chemokine Receptor (CCR5). In particular, the invention encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to a polypeptide or polypeptide fragment or variant of human G-protein Chemokine Receptor (CCR5) such as those of SEQ ID NO:2 or of the polypeptide encoded by the deposited clone.

The present invention relates to methods and compositions for preventing, treating or ameliorating a disease or disorder comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof. In specific embodiments, the present invention relates to methods and compositions for preventing, treating or ameliorating a disease or disorder associated with G-protein Chemokine Receptor (CCR5) function or G-protein

Chemokine Receptor (CCR5) ligand function or aberrant G-protein Chemokine Receptor (CCR5) or G-protein Chemokine Receptor (CCR5) ligand expression, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof. In highly preferred embodiments, the present invention relates to antibody-based methods and compositions for preventing, treating or ameliorating HIV infection and/or conditions associated with HIV infection. Other diseases and disorders which can be treated, prevented or ameliorated with the antibodies of the invention include, but are not limited to, immune disorders (*e.g.*, autoimmune disorders such as multiple sclerosis, Grave's disease, and rheumatoid arthritis), neurodegenerative disorders (*e.g.*, Alzheimer's disease) inflammatory disorders (*e.g.*, asthma, allergic disorders, or inflammatory kidney diseases such as glomerulonephritis), infectious diseases (*e.g.*, Hepatitis infections, herpes viral infections, and other viral infections) and proliferative disorders.

The present invention also encompasses methods and compositions for detecting, diagnosing, or prognosing diseases or disorders comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof. In specific embodiments, the present invention also encompasses methods and compositions for detecting, diagnosing, or prognosing diseases or disorders associated with G-protein Chemokine Receptor (CCR5) function or G-protein Chemokine Receptor (CCR5) ligand function or aberrant G-protein Chemokine Receptor (CCR5) or G-protein Chemokine Receptor (CCR5) ligand expression, comprising administering to an animal, preferably a human, an effective amount of one or more antibodies or fragments or variants thereof, or related molecules, that immunospecifically bind to G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof. In highly preferred embodiments, the present invention relates to antibody-based methods and compositions for detecting, diagnosing, or prognosing HIV infection and/or conditions associated with HIV infection. Other diseases and disorders which can be detected, diagnosed, or prognosed with the antibodies of the invention include, but are not limited to, immune disorders (*e.g.*, autoimmune disorders such as multiple sclerosis, Grave's disease, and rheumatoid arthritis), neurodegenerative disorders (*e.g.*, Alzheimer's disease) inflammatory (*e.g.*, asthma, allergic disorders, or

inflammatory kidney diseases such as glomerulonephritis), infectious diseases (e.g., Hepatitis infections, herpes viral infections, and other viral infections) and proliferative disorders.

Another embodiment of the present invention includes the use of the antibodies of the invention as a diagnostic tool to monitor the expression of G-protein Chemokine Receptor (CCR5) expression on cells.

The present invention also encompasses cell lines that express antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5) polypeptide (e.g., SEQ ID NO:2, or the polypeptide encoded by the deposited clone).

Further, the present invention encompasses the polynucleotides encoding the antibodies expressed by such cell lines, as well as the amino acid sequences encoding the antibodies expressed by these cell lines. Molecules comprising, or alternatively consisting of, fragments or variants of these antibodies (e.g., heavy chains, VH domains, VH CDRs, light chains, VL domains, or VL CDRs having an amino acid sequence of any one of those expressed by an antibody-expressing cell line of the invention, that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules that encode these antibodies and/or molecules. In highly preferred embodiments, the present invention encompasses antibodies, or fragments or variants thereof, that bind to the extracellular regions/domains of a G-protein Chemokine Receptor (CCR5) or a fragment and variant thereof.

The present inventors have generated hybridoma cell lines that express antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5) polypeptide (e.g., SEQ ID NO:2 or the polypeptide encoded by the deposited clone). Thus, the invention encompasses these cell lines, listed in Table 2 below which were deposited with the American Type Culture Collection ("ATCC") on the dates listed in Table 2 and given the ATCC Deposit Numbers identified in Table 2. The ATCC is located at 10801 University Boulevard, Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for Purposes of Patent Procedure.

Further, the present invention encompasses the polynucleotides encoding the antibodies expressed by these cell lines, as well as the amino acid sequences encoding the antibodies expressed by these cell lines. Molecules comprising, or alternatively consisting of, fragments or variants of these antibodies (e.g., heavy chains, VH domains, VH CDRs,

light chains, VL domains, or VL CDRs having an amino acid sequence of any one of those expressed by one or more cell lines referred to in Table 2), that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules that encode these antibodies and/or molecules. In highly preferred embodiments, the present invention encompasses antibodies, or fragments or variants thereof, that bind to the extracellular regions/domains of a G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof.

The present invention also provides antibodies that bind a G-protein Chemokine Receptor (CCR5) polypeptide wherein said antibodies are coupled to a detectable label, such as an enzyme, a fluorescent label, a luminescent label, or a bioluminescent label. The present invention also provides antibodies that bind a G-protein Chemokine Receptor (CCR5) polypeptide wherein said antibodies are coupled to a therapeutic or cytotoxic agent. The present invention also provides antibodies that bind a G-protein Chemokine Receptor (CCR5) polypeptide wherein said antibodies are coupled to a radioactive material.

The present invention further provides antibodies that inhibit or abolish the ability of HIV to bind to, enter into/fuse with (infect), and/or replicate in G-protein Chemokine Receptor (CCR5) expressing cells. In highly preferred embodiments of the present invention, anti-G-protein Chemokine Receptor (CCR5) antibodies of the present invention are used to treat, prevent or ameliorate HIV infection and/or conditions associated with HIV infection. In other highly preferred embodiments, anti-G-protein Chemokine Receptor (CCR5) antibodies of the present invention are administered to an individual alone or in combination with other therapeutic compounds, especially anti-retroviral agents, to treat, prevent or ameliorate HIV infection and/or conditions associated with HIV infection.

The present invention also provides antibodies that bind a G-protein Chemokine Receptor (CCR5) polypeptide and that act as either G-protein Chemokine Receptor (CCR5) agonists or G-protein Chemokine Receptor (CCR5) antagonists. In specific embodiments, the antibodies of the invention stimulate chemotaxis of G-protein Chemokine Receptor (CCR5) expressing cells. In other specific embodiments, the antibodies of the invention inhibit G-protein Chemokine Receptor (CCR5) ligand binding to a G-protein Chemokine Receptor (CCR5). In other specific embodiments, the antibodies of the invention upregulate G-protein Chemokine Receptor (CCR5) expression.

The present invention also provides antibodies that downregulate G-protein Chemokine Receptor (CCR5) expression. In still other specific embodiments, the anti-G-protein Chemokine Receptor (CCR5) antibodies of the invention downregulate G-protein Chemokine Receptor (CCR5) expression by promoting G-protein Chemokine Receptor (CCR5) internalization.

The present invention further provides antibodies that inhibit or abolish the binding of a G-protein Chemokine Receptor (CCR5) ligand, (e.g., MIP1-beta MIP-1alpha, MCP-1, MCP-2, MCP-3, MCP-4, RANTES, and Eotaxin), to G-protein Chemokine Receptor (CCR5) expressing cells.

The antibodies of the present invention may be used for diagnostic or therapeutic purposes. In one embodiment, antibodies of the invention are used to diagnose a susceptibility to HIV infection, and more particularly to diagnose susceptibility to CCR5 tropic strains of HIV. Because CCR5 acts as a cell-surface receptor for HIV, by which HIV initiates its entry into the cell, individuals who have low or absent CCR5 expression, as determined using an antibody of the invention are less susceptible to HIV infection. In another embodiment, antibodies of the invention may be administered therapeutically or prophylactically to treat or prevent HIV infection. In yet another embodiment, antibodies of the invention may be administered therapeutically or prophylactically to treat or prevent graft rejection.

The present invention also provides for a nucleic acid molecule(s), generally isolated, encoding an antibody (including molecules, such as scFvs, VH domains, or VL domains, that comprise, or alternatively consist of, an antibody fragment or variant thereof) of the invention. The present invention also provides a host cell transformed with a nucleic acid molecule encoding an antibody (including molecules, such as scFvs, VH domains, or VL domains, that comprise, or alternatively consist of, an antibody fragment or variant thereof) of the invention and progeny thereof. The present invention also provides a method for the production of an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof) of the invention. The present invention further provides a method of expressing an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof) of the invention from a nucleic acid molecule. These and other aspects of the invention are described in further detail below.

In another embodiment, the present invention provides vaccines comprising, or alternatively consisting of, G-protein Chemokine Receptor (CCR5) polynucleotides or polypeptides or fragments, variants or derivatives thereof.

In accordance with another aspect of the present invention there are provided methods of screening for compounds which bind to and activate or inhibit activation of the G-protein Chemokine Receptor (CCR5) polypeptides of the present invention.

In accordance with still another embodiment of the present invention there are provided processes of administering compounds to a host which bind to and activate the receptor polypeptide of the present invention which are useful in stimulating haematopoiesis, wound healing, coagulation, angiogenesis, to treat solid tumors, chronic infections, leukemia, T-cell mediated auto-immune diseases, parasitic infections, psoriasis, and to stimulate growth factor activity.

In accordance with another aspect of the present invention there is provided a method of administering the receptor polypeptides of the present invention via gene therapy to treat conditions related to underexpression of the polypeptides or underexpression of a ligand for the G-protein Chemokine Receptor (CCR5) polypeptide.

In accordance with still another embodiment of the present invention there are provided processes of administering compounds to a host which bind to and inhibit activation of the receptor polypeptides of the present invention which are useful in the prevention and/or treatment of allergy, atherogenesis, anaphylaxis, malignancy, chronic and acute inflammation, histamine and IgE-mediated allergic reactions, prostaglandin-independent fever, bone marrow failure, silicosis, sarcoidosis, rheumatoid arthritis, shock and hyper-eosinophilic syndrome.

In accordance with yet another aspect of the present invention, there are provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to the polynucleotide sequences of the present invention.

In accordance with still another aspect of the present invention, there are provided diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences encoding such polypeptides and for detecting an altered level of the soluble form of the receptor polypeptides.

In accordance with yet a further aspect of the present invention, there are provided processes for utilizing such receptor polypeptides, or polynucleotides encoding such

polypeptides, for in vitro purposes related to scientific research, synthesis of DNA and manufacture of DNA vectors.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

Brief Description of the Drawings

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

FIG. 1 shows the DNA sequence and the corresponding deduced amino acid sequence of the G-protein coupled receptor of the present invention. The standard one-letter abbreviation for amino acids is used. Sequencing was performed using a 373 Automated DNA sequencer (Applied Biosystems, Inc.).

FIG. 2 illustrates an amino acid alignment of the G-protein Chemokine Receptor (CCR5) of the present invention and the human MCP-1 receptor (SEQ ID NO:9). This figure shows the regions of identity between the amino acid sequence of the G-protein Chemokine Receptor (CCR5) protein and the translation product of the human MCP-1 receptor A (MCP-1 RA) (SEQ ID NO:9), determined by BLAST analysis. Identical amino acids between the two polypeptides are indicated by lines, while highly conservative amino acid are indicated by colons and conservative amino acids are indicated by periods. By examining the regions of identical, highly conserved and conserved amino acids, the skilled artisan can readily identify conserved domains between the two polypeptides. These conserved domains are preferred embodiments of the present invention.

FIG. 3 shows an analysis of the G-protein Chemokine Receptor (CCR5) amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, and all were generated using the default settings. In the "Antigenic Index or Jameson-Wolf" graph, the positive peaks indicate locations of the highly antigenic regions of the G-protein Chemokine Receptor (CCR5) protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained. The domains defined by these graphs are contemplated by the present invention.

The data presented in FIG. 3 are also represented in tabular form in Table 1. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in FIG. 3, and Table 1: "Res": amino acid residue of SEQ ID NO:2 and FIG. 1; "Position": position of the corresponding residue within SEQ ID NO:2 and FIG. 1; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta, Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

FIG. 4 shows the polynucleotide and amino acid sequences of the VH (SEQ ID NOS:71 and 72, respectively) and VL (SEQ ID NOS:73 and 74, respectively) domains of anti-CCR5 antibody 36F11. Each CDR is indicated by a line above the nucleotide sequence. See also, Table 6.

FIG. 5 shows the polynucleotide and amino acid sequences of the VH (SEQ ID NOS:75 and 76, respectively) and VL (SEQ ID NOS:77 and 78, respectively) domains of anti-CCR5 antibody 43E2. Each CDR is indicated by a line above the nucleotide sequence. See also, Table 6.

FIG. 6 shows the polynucleotide and amino acid sequences of the VL domain (SEQ ID NOS:79 and 80, respectively) of anti-CCR5 antibody AK38181. Each CDR is indicated by a line above the nucleotide sequence. See also, Table 6.

FIG. 7A shows a saturation binding assay in which Europium (Eu) labeled 43E2 antibody of FIG. 5 binds to CCR5 expressed on the surface of a human T cell leukemia cell line, CEM.NKR. FIG. 7B shows a saturation binding assay in which an Eu labeled 43E2/AK38181 antibody is being assessed. This antibody comprises a heavy chain with the VH domain of SEQ ID NO:76 fused to the IgG4 constant domain (SEQ ID NO:82) and a light chain comprising the VL domain of SEQ ID NO:80 (from FIG. 6 above) fused to the human kappa constant domain (SEQ ID NO:83). The dissociation constant (Kd) is shown in the legend for each figure. See also, Example 67.

FIGS 8A-C show the effect of 43E2, 43E2/AK38181 or non-CCR5 specific IgG4 isotype control antibodies on the entry of a CCR5-tropic human immunodeficiency virus

type 1 strain (HIV-1-JRFL) into CD8-depleted, activated T-cells isolated from healthy human donors. The data points represent the mean of triplicate measurements $\pm$ standard deviation. The concentration of antibody at which the data lines cross the dashed line represents the IC₅₀.

Detailed Description

In accordance with an aspect of the present invention, there is provided an isolated nucleic acid (polynucleotide) which encodes for the mature polypeptide having the deduced amino acid sequence of FIG. 1 (SEQ ID NO:2) or for the mature polypeptide encoded by the clone deposited as ATCC Deposit No. 97183 on June 1, 1995. A sample of the deposited clone, which contains the open reading frame of the G-protein Chemokine Receptor (CCR5), has been obtained from the ATCC and has been resequenced. The sequence data from the resequenced clone is shown in SEQ ID NO:21 and 22. SEQ ID NO:21 differs from SEQ ID NO:1 at 5 positions (nucleotides 320, 433, 442, 646, and 1289 of SEQ ID NO:1) SEQ ID NO:22 differs from SEQ ID NO:2 at 5 positions (amino acid residues 21, 59, 62, 130, and 344).

The polynucleotide of this invention was discovered in a genomic library derived from human monocytes. It is structurally related to the G-protein-coupled receptor family. It contains an open reading frame encoding a protein of 352 amino acid residues. The protein exhibits the highest degree of homology to a human MCP-1 receptor (SEQ ID NO:9) with 70.1% identity and 82.9 % similarity over a 347 amino acid stretch.

Polynucleotides of the invention include, but are not limited to, the nucleotide sequence of SEQ ID NO:1, the nucleotide sequence of the HDG NR10 deposited clone (ATCC Deposit Number 97183), the nucleotide sequence of SEQ ID NO:21), and/or fragments, variants or derivatives thereof.

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the mature polypeptide may be identical to the coding sequence shown in FIG. 1 (SEQ ID NO:1) or that of the deposited clone or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same mature polypeptide as the DNA of FIG. 1 (SEQ ID NO:1) or the deposited clone.

The polynucleotide which encodes for the mature polypeptide of FIG. 1 or for the mature polypeptide encoded by the deposited clone may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence such as a transmembrane (TM) or intra-cellular domain; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptide having the deduced amino acid sequence of FIG. 1 or the polypeptide encoded by the deposited clone. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same mature polypeptide as shown in FIG. 1 (SEQ ID NO:2) or the same mature polypeptide encoded by the deposited clone as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide of FIG. 1 (SEQ ID NO:2) or the polypeptide encoded by the deposited clone. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in FIG. 1 (SEQ ID NO:1) or of the coding sequence of the deposited clone. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The polynucleotides may also encode for a soluble form of the G-protein Chemokine Receptor (CCR5) polypeptide which is the extracellular portion of the polypeptide which has been cleaved from the TM and intracellular domain of the full-length polypeptide of the present invention.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence which allows for purification of the polypeptide of

the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptide fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., *et al.*, *Cell*, 37:767 (1984)).

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

Fragments of the full length gene of the present invention may be used as a hybridization probe for a cDNA library to isolate the full length cDNA and to isolate other cDNAs which have a high sequence similarity to the gene or similar biological activity. Probes of this type preferably have at least 30 bases and may contain, for example, 50 or more bases. The probe may also be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete gene including regulatory and promoter regions, exons, and introns. An example of a screen comprises isolating the coding region of the gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of human cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide encoded by the DNAs of FIG. 1 (SEQ ID NO:1) or the deposited clone.

Alternatively, the polynucleotide may have at least 20 bases, preferably 30 bases, and more preferably at least 50 bases which hybridize to a polynucleotide of the present

invention and which has an identity thereto, as hereinabove described, and which may or may not retain activity. For example, such polynucleotides may be employed as probes for the polynucleotide of SEQ ID NO:1 or of the deposited clone, for example, for recovery of the polynucleotide or as a diagnostic probe or as a PCR primer.

Thus, the present invention is directed to polynucleotides having at least a 70% identity, preferably at least 90% and more preferably at least a 95% identity to a polynucleotide which encodes the polypeptide of SEQ ID NO:2 or that encoded by the deposited clone as well as fragments thereof, which fragments have at least 30 bases and preferably at least 50 bases and to polypeptides encoded by such polynucleotides.

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to a G-protein Chemokine Receptor (CCR5) polypeptide which has the deduced amino acid sequence of FIG. 1 (SEQ ID NO:2) or which has the amino acid sequence encoded by the deposited clone (SEQ ID NO:22), as well as fragments, analogs and derivatives of such polypeptide.

The terms "fragment," "derivative" and "analog" when referring to the polypeptide of FIG. 1 or that encoded by the deposited clone, means a polypeptide which either retains substantially the same biological function or activity as such polypeptide, i.e. functions as a G-protein Chemokine Receptor (CCR5), or retains the ability to bind the ligand or the receptor even though the polypeptide does not function as a G-protein Chemokine Receptor (CCR5), for example, a soluble form of the receptor. An analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide of FIG. 1 (SEQ ID NO: 2) or that encoded by the deposited clone may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide for purification of the polypeptide or (v) one in which a fragment of the polypeptide is soluble, i.e. not membrane bound, yet still binds ligands to the membrane bound receptor. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The polypeptides of the present invention include the polypeptide of SEQ ID NO:2 (in particular the mature polypeptide) or that encoded by the deposited clone as well as polypeptides which have at least 70% similarity (preferably a 70% identity) to the polypeptide of SEQ ID NO:2) or to that encoded by the deposited clone and more preferably a 90% similarity (more preferably a 90% identity) to the polypeptide of SEQ ID NO:2) or to that encoded by the deposited clone and still more preferably a 95% similarity (still more preferably a 90% identity) to the polypeptide of SEQ ID NO:2 and to portions of such polypeptide with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and conserved amino acid substitutes thereto of the polypeptide to the sequence of a second polypeptide.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis, therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region "leader and trailer" as well as intervening sequences (introns) between individual coding segments (exons).

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The polypeptides of the present invention include the polypeptide of SEQ ID NO:2 or that encoded by the deposited clone (in particular the mature polypeptide) as well as polypeptides which have at least 70% similarity (preferably at least 70% identity) and more preferably at least 90% similarity (more preferably at least 90% identity) and still more preferably at least 95% similarity (still more preferably at least 95% identity) to the polypeptide of SEQ ID NO:2) or to that encoded by the deposited clone and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral

particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the present invention. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the *E. coli*, *lac* or *trp*, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as *E. coli*, *Streptomyces*, *Salmonella typhimurium*; fungal cells, such as yeast;

insect cells such as *Drosophila* and *Spodoptera Sf9*; animal cells such as CHO, COS or Bowes melanoma; adenovirus; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation. (Davis, L., *et al.*, *Basic Methods in Molecular Biology*, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, *et al.*, *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α -factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well known to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell* 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The G-protein Chemokine Receptor (CCR5) polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to human disease.

The G-protein Chemokine Receptor (CCR5) of the present invention may be employed in a process for screening for compounds which activate (agonists) or inhibit activation (antagonists) of the receptor polypeptide of the present invention.

In general, such screening procedures involve providing appropriate cells which express the receptor polypeptide of the present invention on the surface thereof. Such cells include cells from mammals, yeast, drosophila or *E. coli*. In particular, a polynucleotide encoding the receptor of the present invention is employed to transfect cells to thereby express the G-protein Chemokine Receptor (CCR5). The expressed receptor is then contacted with a test compound to observe binding, stimulation or inhibition of a functional response.

One such screening procedure involves the use of melanophores which are transfected to express the G-protein Chemokine Receptor (CCR5) of the present invention. Such a screening technique is described in PCT WO 92/01810 published February 6, 1992.

Thus, for example, such assay may be employed for screening for a compound which inhibits activation of the receptor polypeptide of the present invention by contacting the melanophore cells which encode the receptor with both the receptor ligand and a compound to be screened. Inhibition of the signal generated by the ligand indicates that a compound is a potential antagonist for the receptor, i.e., inhibits activation of the receptor.

The screen may be employed for determining a compound which activates the receptor by contacting such cells with compounds to be screened and determining whether such compound generates a signal, i.e., activates the receptor.

Other screening techniques include the use of cells which express the G-protein Chemokine Receptor (CCR5) (for example, transfected CHO cells) in a system which measures extracellular pH changes caused by receptor activation, for example, as described in *Science* 246:181-296 (October 1989). For example, compounds may be contacted with a cell which expresses the receptor polypeptide of the present invention and a second messenger response, e.g. signal transduction or pH changes, may be measured to determine whether the potential compound activates or inhibits the receptor.

Another such screening technique involves introducing RNA encoding the G-protein Chemokine Receptor (CCR5) into *Xenopus* oocytes to transiently express the receptor. The receptor oocytes, may then be contacted with the receptor ligand and a compound to be screened, followed by detection of inhibition or activation of a calcium signal in the case of screening for compounds which are thought to inhibit activation of the receptor.

Another screening technique involves expressing the G-protein Chemokine Receptor (CCR5) in which the receptor is linked to a phospholipase C or D. As representative examples of such cells, there may be mentioned endothelial cells, smooth muscle cells, embryonic kidney cells, etc. The screening may be accomplished as hereinabove described by detecting activation of the receptor or inhibition of activation of the receptor from the phospholipase second signal.

Another method involves screening for compounds which inhibit activation of the receptor polypeptide of the present invention antagonists by determining inhibition of binding of labeled ligand to cells which have the receptor on the surface thereof. Such a method involves transfecting a eukaryotic cell with DNA encoding the G-protein Chemokine Receptor (CCR5) such that the cell expresses the receptor on its surface and contacting the cell with a compound in the presence of a labeled form of a known ligand. The ligand can be labeled, e.g., by radioactivity. The amount of labeled ligand bound to the receptors is measured, e.g., by measuring radioactivity of the receptors. If the compound binds to the receptor as determined by a reduction of labeled ligand which binds to the receptors, the binding of labeled ligand to the receptor is inhibited.

An antibody, or in some cases an oligopeptide, may activate a G-protein Chemokine Receptor (CCR5) of the present invention, by binding to the G-protein Chemokine Receptor (CCR5) and initiating second messenger response. Antibodies include anti-idiotypic antibodies which recognize unique determinants generally

associated with the antigen-binding site of an antibody. Potential agonist compounds also include proteins which are closely related to the ligand of the G-protein Chemokine Receptor (CCR5), e.g., a fragment of the ligand.

An antibody, or in some cases an oligopeptide, may antagonize a G-protein Chemokine Receptor (CCR5) of the present invention, by binding to the G-protein Chemokine Receptor (CCR5) but failing to elicit a second messenger response such that the activity of the G-protein Chemokine Receptor (CCR5) is prevented. Antibodies include anti-idiotypic antibodies which recognize unique determinants generally associated with the antigen-binding site of an antibody. Potential antagonist compounds also include proteins which are closely related to the ligand of the G-protein Chemokine Receptor (CCR5), e.g., a fragment of the ligand that has lost biological function and elicits no response when binding to the G-protein Chemokine Receptor (CCR5).

An antisense construct prepared through the use of antisense technology, may be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix; see Lee *et al.*, *Nucl. Acids Res.* 6:3073 (1979); Cooney *et al.*, *Science* 241:456 (1988); and Dervan *et al.*, *Science* 251:1360 (1991)), thereby preventing transcription and the production of G-protein Chemokine Receptor (CCR5). The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of mRNA molecules into G-protein coupled receptor (antisense - Okano, *J. Neurochem.* 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of G-protein Chemokine Receptor (CCR5).

A small molecule which binds to the G-protein Chemokine Receptor (CCR5), making it inaccessible to ligands such that normal biological activity is prevented, for example small peptides or peptide-like molecules, may also be used to inhibit activation of the receptor polypeptide of the present invention.

A soluble form of the G-protein Chemokine Receptor (CCR5), e.g. a fragment of the receptors, may be used to inhibit activation of the receptor by binding to the ligand to a polypeptide of the present invention and preventing the ligand from interacting with membrane bound G-protein Chemokine Receptor (CCR5).

The compounds which bind to and activate the G-protein Chemokine Receptor (CCR5) of the present invention may be employed to stimulate haematopoiesis, wound healing, coagulation, angiogenesis, to treat solid tumors, chronic infections, leukemia, T-cell mediated auto-immune diseases, parasitic infections, psoriasis, and to stimulate growth factor activity.

The compounds which bind to and inhibit the G-protein Chemokine Receptor (CCR5) of the present invention may be employed to treat allergy, atherogenesis, anaphylaxis, malignancy, chronic and acute inflammation, histamine and IgE-mediated allergic reactions, prostaglandin-independent fever, bone marrow failure, silicosis, sarcoidosis, rheumatoid arthritis, shock and hyper-eosinophilic syndrome.

The compounds may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the compound and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the compounds of the present invention may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, the pharmaceutical compositions will be administered in an amount of at least about 10 µg/kg body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day. In most cases, the dosage is from about 10 µg/kg to

about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The G-protein Chemokine Receptor (CCR5) polypeptides and antagonists or agonists which are polypeptides, may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells *in vivo* after combination with a suitable delivery vehicle.

Retroviruses from which the retroviral plasmid vectors hereinabove mentioned may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. In one embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia virus.

The vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, *et al.*, *Biotechniques* 7:980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β -actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters.

The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The nucleic acid sequence encoding the polypeptide of the present invention is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoA1 promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs hereinabove described); the β -actin promoter; and human growth hormone promoters. The promoter also may be the native promoter which controls the genes encoding the polypeptides.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, ψ -2, ψ -AM, PA12, T19-14X, VT-19-17-H2, ψ CRE, ψ CRIP, GP+E-86, GP+envAM12, and DAN cell lines as described in Miller, *Human Gene Therapy* 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO_4 precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

The present invention also provides a method for determining whether a ligand not known to be capable of binding to a G-protein Chemokine Receptor (CCR5) can bind to such receptor which comprises contacting a mammalian cell which expresses a G-protein

Chemokine Receptor (CCR5) with the ligand under conditions permitting binding of ligands to the G-protein Chemokine Receptor (CCR5), detecting the presence of a ligand which binds to the receptor and thereby determining whether the ligand binds to the G-protein Chemokine Receptor (CCR5). The systems hereinabove described for determining agonists and/or antagonists may also be employed for determining ligands which bind to the receptor.

This invention also provides a method of detecting expression of a G-protein Chemokine Receptor (CCR5) polypeptide of the present invention on the surface of a cell by detecting the presence of mRNA coding for the receptor which comprises obtaining total mRNA from the cell and contacting the mRNA so obtained with a nucleic acid probe comprising a nucleic acid molecule of at least 10 nucleotides capable of specifically hybridizing with a sequence included within the sequence of a nucleic acid molecule encoding the receptor under hybridizing conditions, detecting the presence of mRNA hybridized to the probe, and thereby detecting the expression of the receptor by the cell.

The present invention also provides a method for identifying receptors related to the receptor polypeptides of the present invention. These related receptors may be identified by homology to a G-protein Chemokine Receptor (CCR5) polypeptide of the present invention, by low stringency cross hybridization, or by identifying receptors that interact with related natural or synthetic ligands and or elicit similar behaviors after genetic or pharmacological blockade of the chemokine receptor polypeptides of the present invention.

Fragments of the genes may be used as a hybridization probe for a cDNA library to isolate other genes which have a high sequence similarity to the genes of the present invention, or which have similar biological activity. Probes of this type are at least 20 bases, preferably at least 30 bases and most preferably at least 50 bases or more. The probe may also be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete gene of the present invention including regulatory and promoter regions, exons and introns. An example of a screen of this type comprises isolating the coding region of the gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the genes of the present invention are used to screen a library of human cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The present invention also contemplates the use of the genes of the present invention as a diagnostic, for example, some diseases result from inherited defective genes. These genes can be detected by comparing the sequences of the defective gene with that of a normal one. Subsequently, one can verify that a "mutant" gene is associated with abnormal receptor activity. In addition, one can insert mutant receptor genes into a suitable vector for expression in a functional assay system (e.g., colorimetric assay, expression on MacConkey plates, complementation experiments, in a receptor deficient strain of HEK293 cells) as yet another means to verify or identify mutations. Once "mutant" genes have been identified, one can then screen population for carriers of the "mutant" receptor gene.

Individuals carrying mutations in the gene of the present invention may be detected at the DNA level by a variety of techniques. Nucleic acids used for diagnosis may be obtained from a patient's cells, including but not limited to such as from blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki, *et al.*, *Nature* 324:163-166 (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complimentary to the nucleic acid of the instant invention can be used to identify and analyze mutations in the gene of the present invention. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled RNA of the invention or alternatively, radiolabeled antisense DNA sequences of the invention. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures. Such a diagnostic would be particularly useful for prenatal or even neonatal testing.

Sequence differences between the reference gene and "mutants" may be revealed by the direct DNA sequencing method. In addition, cloned DNA segments may be used as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequence primer is used with double stranded PCR product or a single stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by an automatic sequencing procedure with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alterations in the electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Sequences changes at specific locations may also be revealed by nucleus protection assays, such RNase and S1 protection or the chemical cleavage method (e.g. Cotton, *et al.*, *PNAS, USA* 85:4397-4401 (1985)).

In addition, some diseases are a result of, or are characterized by changes in gene expression which can be detected by changes in the mRNA. Alternatively, the genes of the present invention can be used as a reference to identify individuals expressing a decrease of functions associated with receptors of this type.

The present invention also relates to a diagnostic assay for detecting altered levels of soluble forms of the G-protein Chemokine Receptor (CCR5) polypeptides of the present invention in various tissues. Assays used to detect levels of the soluble receptor polypeptides in a sample derived from a host are well known to those of skill in the art and include radioimmunoassays, competitive-binding assays, Western blot analysis and preferably as ELISA assay.

An ELISA assay initially comprises preparing an antibody specific to antigens of the G-protein Chemokine Receptor (CCR5) polypeptides, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter antibody is attached a detectable reagent such as radioactivity, fluorescence or in this example a horseradish peroxidase enzyme. A sample is now removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any G-protein Chemokine Receptor (CCR5) proteins attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to G-protein Chemokine Receptor (CCR5) proteins. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of G-protein Chemokine Receptor (CCR5) proteins present in a given volume of patient sample when compared against a standard curve.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the clone. Computer analysis of the DNA of the deposited clone is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-DNA libraries.

Fluorescence *in situ* hybridization (FISH) of a DNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with DNA as short as 50 or 60 bases. For a review of this technique, see Verma *et al.*, *Human Chromosomes: a Manual of Basic Techniques*, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man* (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal

region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, *Nature* 256:495-497 (1975)), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* 4:72 (1983)), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985), pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide

products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 µg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 µl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 µg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, *Nucleic Acids Res.* 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., *et al.*, *Id.*, p. 146). Unless otherwise

provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 µg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., *Virology* 52:456-457 (1973).

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

In the present invention, a "secreted" or "soluble" G-protein Chemokine Receptor (CCR5) protein refers to a protein capable of being directed to the ER, secretory vesicles, or the extracellular space as a result of a signal sequence, as well as a G-protein Chemokine Receptor (CCR5) protein released into the extracellular space without necessarily containing a signal sequence. If the G-protein Chemokine Receptor (CCR5) secreted protein is released into the extracellular space, the G-protein Chemokine Receptor (CCR5) secreted protein can undergo extracellular processing to produce a "mature" G-protein Chemokine Receptor (CCR5) protein. Release into the extracellular space can occur by many mechanisms, including exocytosis and proteolytic cleavage. Examples of secreted or soluble G-protein Chemokine Receptor (CCR5) protein include fragments comprising, or alternatively consisting of, portions of the G-protein Chemokine Receptor (CCR5) described herein. Preferred secreted or soluble fragments comprise an extracellular loop, an intracellular loop, the N-terminal extracellular domain, or the C-terminal intracellular domain, or fragments thereof. Additional preferred secreted or soluble fragments comprise an epitope of the the G-protein Chemokine Receptor (CCR5), such as described herein.

As used herein, a G-protein Chemokine Receptor (CCR5) "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:1 or the G-protein

Chemokine Receptor DNA contained within the clone deposited with the ATCC. For example, the G-protein Chemokine Receptor (CCR5) polynucleotide can contain the nucleotide sequence of the full length genomic sequence, including the 5' and 3' untranslated sequences, the coding region, with or without the signal sequence, the secreted protein coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a G-protein Chemokine Receptor (CCR5) "polypeptide" refers to a molecule having the translated amino acid sequence generated from the polynucleotide as broadly defined.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the G-protein Chemokine Receptor (CCR5) gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

A representative clone containing the open reading frame of the sequence for SEQ ID NO:1 was deposited with the American Type Culture Collection ("ATCC") on June 1, 1995, and was given the ATCC Deposit Number 97183. The ATCC is located at 10801 University Boulevard, Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for purposes of patent procedure.

A G-protein Chemokine Receptor (CCR5) "polynucleotide" also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:1, the complement thereof, or the DNA within the deposited clone. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also contemplated are nucleic acid molecules that hybridize to the G-protein Chemokine Receptor (CCR5) polynucleotides under lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH₂PO₄; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA⁺ sequences (such as any 3' terminal polyA⁺ tract of a DNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The G-protein Chemokine Receptor (CCR5) polynucleotide can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, G-protein Chemokine Receptor (CCR5) polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the G-protein Chemokine Receptor (CCR5) polynucleotides can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. G-protein Chemokine Receptor (CCR5)

polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

G-protein Chemokine Receptor (CCR5) polypeptides can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The G-protein Chemokine Receptor (CCR5) polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the G-protein Chemokine Receptor (CCR5) polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given G-protein Chemokine Receptor (CCR5) polypeptide. Also, a given G-protein Chemokine Receptor (CCR5) polypeptide may contain many types of modifications. G-protein Chemokine Receptor (CCR5) polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic G-protein Chemokine Receptor (CCR5) polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993);

POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

"SEQ ID NO:1" refers to a G-protein Chemokine Receptor (CCR5) polynucleotide sequence while "SEQ ID NO:2" refers to a G-protein Chemokine Receptor (CCR5) polypeptide sequence.

A G-protein Chemokine Receptor (CCR5) polypeptide "having biological activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a G-protein Chemokine Receptor (CCR5) polypeptide, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the G-protein Chemokine Receptor (CCR5) polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the G-protein Chemokine Receptor (CCR5) polypeptide (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the G-protein Chemokine Receptor (CCR5) polypeptide.)

G-protein Chemokine Receptor (CCR5) Polynucleotides and Polypeptides

Clone HDGNR10 was isolated from a human monocyte genomic DNA library. This clone contains the entire coding region identified as SEQ ID NO:2. The deposited clone contains a DNA insert having a total of 1414 nucleotides, which encodes a predicted open reading frame of 352 amino acid residues. (See FIG. 1.) The open reading frame begins at a N-terminal methionine located at nucleotide position 259, and ends at the last triplet coding for an amino acid at nucleotide position 1314. The stop codon is at positions 1315-1317.

Subsequent expression analysis also showed G-protein Chemokine Receptor (CCR5) expression in macrophages, including immature dendritic cells such as Langerhans cells, and T cells, including Th0 and Th1 effector cells, a pattern consistent with immune system-specific expression. G-protein Chemokine Receptor (CCR5) has also been detected in microglia, astrocytes, neurons, and vascular endothelial cells of the central nervous system (CNS). G-protein Chemokine Receptor (CCR5) is also expressed

in monocytes and T cells in the synovial fluid of rheumatoid arthritis patients, and has also been implicated in other forms of arthritis.

G-protein Coupled Chemokine Receptors. Using BLAST analysis, SEQ ID NO:2 was found to be homologous to members of the G-Protein COUPLED Chemokine Receptor family. Particularly, SEQ ID NO:2 contains domains homologous to the translation product of the MonoMac 6 mRNA for human MCP-1 receptor (MCP-1R) A (FIG. 2) (GenBank Accession No. U03882; SEQ ID NO:9), including the conserved transmembrane domain containing seven transmembrane segments characteristic of the G-protein coupled receptor family, which begins with amino acid 37 of SEQ ID NO:2 or the polypeptide encoded by the deposited clone. G-protein Chemokine Receptor (CCR5) also includes the DRY motif, which is known to be required for signal transduction, found in many G-protein coupled receptors immediately following the third transmembrane segment. Because MCP-1R is thought to be important in the immune system, the homology between MCP-1R and G-protein Chemokine Receptor (CCR5) suggests that G-protein Chemokine Receptor (CCR5) may also be involved in the immune system.

A second MCP-1R sequence has also been isolated which is identical to the MCP-1RA sequence from the 5' untranslated region through the putative seventh transmembrane domain but which contains a different cytoplasmic tail. This second sequence, termed MCP-1RB, appears to be an alternatively spliced version of MCP-1RA. It is described further in U.S. Patent No. 5,707,815.

Domains. Using BLAST analysis, SEQ ID NO:2 was found to be homologous to members of the G-protein Chemokine Receptor (CCR5) family. Particularly, SEQ ID NO:2 contains domains homologous to the translation product of the MonoMac 6 mRNA for human MCP-1 receptor (MCP-1R) A (FIG. 2) (GenBank Accession No. U03882; SEQ ID NO:9), including the following conserved domains: (a) a predicted N-terminal extracellular domain located at about amino acids 1 to 36; (b) a predicted transmembrane domain located at about amino acids 37 to 305; and (c) a predicted C-terminal intracellular domain located at about amino acids 306 to 352. The predicted transmembrane domain includes: seven transmembrane segments at about amino acids 37 to 58 (segment 1), 68 to 88 (segment 2), 103 to 124 (segment 3), 142 to 166 (segment 4), 196 to 223 (segment 5), 236 to 260 (segment 6), and 287 to 305 (segment 7); three intracellular loops at about amino acids 59 to 67 (intracellular loop 1), 125 to 141 (intracellular loop 2), and 224 to 235 (intracellular loop 3); and three extracellular loops at about amino acids 89 to 102

(extracellular loop 1), 167 to 195 (extracellular loop 2), and 261 to 274 (extracellular loop 3). These polypeptide fragments of G-protein Chemokine Receptor (CCR5) as defined above or as encoded by the deposited clone (SEQ ID NO:22) are specifically contemplated in the present invention, as are combinations of these and other regions disclosed herein. Also contemplated are polypeptides which exclude one or more of these domains, segments, and loops. The "loops" are also referred to as "regions," "domains," and "portions" herein and in the art, e.g., extracellular "regions", intracellular "regions", extracellular "domains", and intracellular "domains", extracellular "portions", and intracellular "portions".

SEQ ID NO:1 and the translated SEQ ID NO:2 are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, SEQ ID NO:1 is useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:1 or the DNA contained in the deposited clone. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:2 may be used, for example, to generate antibodies which bind specifically to proteins G-protein Chemokine Receptor.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:1 and the predicted translated amino acid sequence identified as SEQ ID NO:2, but also a sample of plasmid DNA containing a human DNA of G-protein Chemokine Receptor (CCR5) deposited with the ATCC. The nucleotide sequence of the deposited G-protein Chemokine Receptor (CCR5) clone can readily be determined by sequencing the deposited clone in accordance with known methods. The predicted G-protein Chemokine Receptor (CCR5) amino acid sequence can

then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by the deposited clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human G-protein Chemokine Receptor (CCR5) DNA, collecting the protein, and determining its sequence. A sample of the deposited clone, which contains the open reading frame of the G-protein Chemokine Receptor (CCR5), has been obtained from the ATCC and has been resequenced. The sequence data from the resequenced clone is shown in SEQ ID NO:21 and 22. SEQ ID NO:21 differs from SEQ ID NO:1 at 5 positions (nucleotides 320, 433, 442, 646, and 1289 of SEQ ID NO:1) SEQ ID NO:22 differs from SEQ ID NO:2 at 5 positions (amino acid residues 21, 59, 62, 130, and 344).

The present invention also relates to the G-protein Chemokine Receptor (CCR5) gene corresponding to SEQ ID NO:1, SEQ ID NO:2, or the deposited clone. The G-protein Chemokine Receptor (CCR5) gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the G-protein Chemokine Receptor (CCR5) gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:1, SEQ ID NO:2, or a the deposited clone, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The G-protein Chemokine Receptor (CCR5) polypeptides can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The G-protein Chemokine Receptor (CCR5) polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often advantageous to include an additional amino

acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

G-protein Chemokine Receptor (CCR5) polypeptides are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a G-protein Chemokine Receptor (CCR5) polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). G-protein Chemokine Receptor (CCR5) polypeptides also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the G-protein Chemokine Receptor (CCR5) protein.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:1, and/or a clone contained in ATCC deposit 97183. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:2 and/or a polypeptide encoded by the clone contained in ATCC deposit 97183. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:2 and/or a polypeptide sequence encoded by the clone contained in ATCC deposit 97183 are also encompassed by the invention.

Signal Sequences

As described herein, the present invention also encompasses fusions of a signal sequence with the polypeptide of SEQ ID NO:2, and fragments thereof, and/or the polypeptide encoded by the deposited clone, and fragments thereof, to direct secretion of the polypeptide or fragment. Polynucleotides encoding such fusions are also encompassed by the invention.

The present invention also encompasses mature forms of the polypeptide having the sequence of SEQ ID NO:2, and fragments thereof, and/or the polypeptide sequence encoded by the deposited clone, and fragments thereof. Polynucleotides encoding the mature forms (such as, for example, the polynucleotide sequence in SEQ ID NO:1, and fragments thereof, and/or the polynucleotide sequence contained in the deposited clone, and fragments thereof) are also encompassed by the invention.

According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species of the protein. Further, it has long been known that cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide.

Methods for predicting whether a protein has a signal sequence, as well as the cleavage point for that sequence, are available. For instance, the method of McGeoch, *Virus Res.* 3:271-286 (1985), uses the information from a short N-terminal charged region and a subsequent uncharged region of the complete (uncleaved) protein. The method of von Heinje, *Nucleic Acids Res.* 14:4683-4690 (1986) uses the information from the residues surrounding the cleavage site, typically residues -13 to +2, where +1 indicates the amino terminus of the secreted protein. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each of these methods is in the range of 75-80%. (von Heinje, *supra.*) However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

The deduced amino acid sequence of a secreted polypeptide can be analyzed by a computer program called SignalP (Henrik Nielsen et al., *Protein Engineering* 10:1-6 (1997)), which predicts the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Heinje are incorporated.

As one of ordinary skill would appreciate, however, cleavage sites sometimes vary from organism to organism and cannot be predicted with absolute certainty. Cleavage of a heterologous signal sequence in a fusion protein may occur at the junction of the polypeptide sequences or cleavage may occur at a position on either side of the junction. Accordingly, the present invention provides secreted polypeptides having a sequence shown in SEQ ID NO:2, and fragments thereof, which have an N-terminus beginning within 5 residues (i.e., + or - 5 residues) of the predicted cleavage point. Similarly, it is also recognized that in some cases, cleavage of the signal sequence from a secreted protein is not entirely uniform, resulting in more than one secreted species. These polypeptides

and fragments, and the polynucleotides encoding such polypeptides and fragments, are contemplated by the present invention.

Moreover, the signal sequence identified by the above analysis may not necessarily predict the naturally occurring signal sequence. For example, the naturally occurring signal sequence may be further upstream from the predicted signal sequence. However, it is likely that the predicted signal sequence will be capable of directing the secreted protein to the ER. Nonetheless, the present invention provides the mature protein or fragment produced by expression of the polynucleotide sequence of SEQ ID NO:1 or a fragment thereof and/or the polynucleotide sequence contained in the deposited clone or a fragment thereof, in a mammalian cell (e.g., COS cells, as described below). These polypeptides, and the polynucleotides encoding such polypeptides, are contemplated by the present invention.

Polynucleotide and Polypeptide Variants

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:1, the complementary strand thereto, and/or the sequence contained in a deposited clone.

The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:2 and/or encoded by a deposited clone.

"Variant" refers to a polynucleotide or polypeptide differing from the G-protein Chemokine Receptor (CCR5) polynucleotide or polypeptide, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the G-protein Chemokine Receptor (CCR5) polynucleotide or polypeptide.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for example, the nucleotide coding sequence in SEQ ID NO:1 or the complementary strand thereto, the nucleotide coding sequence contained in a deposited clone or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, a nucleotide sequence encoding the polypeptide encoded by the HDG NR10 deposited clone, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower

stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% identical to, for example, the polypeptide sequence shown in SEQ ID NO:2, the polypeptide sequence encoded by the deposited clone, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein).

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the G-protein Chemokine Receptor (CCR5) polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence shown of SEQ ID NO:1, the ORF (open reading frame) of the HDGMR10 DNA in the deposited clone, or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence or polypeptide of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. (1990) 6:237-245.) In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per

each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequences of SEQ ID NO:2 or to the amino acid sequence encoded by the deposited clone can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent

identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The G-protein Chemokine Receptor (CCR5) variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. G-protein Chemokine Receptor (CCR5) polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*).

Naturally occurring G-protein Chemokine Receptor (CCR5) variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given

locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the G-protein Chemokine Receptor (CCR5) polypeptides. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the secreted protein without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes G-protein Chemokine Receptor (CCR5) polypeptide variants which show substantial biological activity. Such variants include

deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity.

The present application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences disclosed herein, (e.g., encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion disclosed below as m-n of SEQ ID NO:2) or which correspond to the polypeptide encoded by the deposited clone, irrespective of whether they encode a polypeptide having G-protein Chemokine Receptor (CCR5) functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having G-protein Chemokine Receptor (CCR5) functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having G-protein Chemokine Receptor (CCR5) functional activity include, inter alia, (1) isolating a G-protein Chemokine Receptor (CCR5) gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the G-protein Chemokine Receptor (CCR5) gene, as described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting G-protein Chemokine Receptor (CCR5) mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having G-protein Chemokine Receptor (CCR5) functional activity. By "a polypeptide having G-protein Chemokine Receptor (CCR5) functional activity" is intended polypeptides exhibiting activity similar, but not necessarily identical, to a functional activity of the G-protein Chemokine Receptor (CCR5) polypeptides of the present invention (e.g., complete (full-length) G-protein Chemokine Receptor (CCR5), mature G-protein Chemokine Receptor (CCR5) and soluble G-protein Chemokine Receptor (CCR5) (e.g., having sequences contained in the extracellular domain or regions of G-protein Chemokine Receptor) as measured, for example, in a particular immunoassay or biological assay. For example, a G-protein Chemokine Receptor (CCR5) functional activity can routinely be measured by determining the ability of a G-protein Chemokine Receptor (CCR5) polypeptide to bind a G-protein Chemokine

Receptor (CCR5) ligand. G-protein Chemokine Receptor (CCR5) functional activity may also be measured by determining the ability of a polypeptide, such as cognate ligand which is free or expressed on a cell surface, to induce cells expressing the polypeptide.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 90%, 95%, 96%, 97%, 98%, or 99% identical to the nucleic acid sequence of the deposited clone, the nucleic acid sequence shown in FIG. 1 (SEQ ID NO:1), or fragments thereof, will encode polypeptides "having G-protein Chemokine Receptor (CCR5) functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having G-protein Chemokine Receptor (CCR5) functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and

Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

For example, site directed changes at the amino acid level of G-protein Chemokine Receptor (CCR5) can be made by replacing a particular amino acid with a conservative amino acid. Preferred conservative mutations of G-protein Chemokine Receptor (CCR5) (SEQ ID NO:2) include: M1 replaced with A, G, I, L, S, T, or V; D2 replaced with E; Y3 replaced with F, or W; Q4 replaced with N; V5 replaced with A, G, I, L, S, T, or M; S6 replaced with A, G, I, L, T, M, or V; S7 replaced with A, G, I, L, T, M, or V; I9 replaced with A, G, L, S, T, M, or V; Y10 replaced with F, or W; D11 replaced with E; I12 replaced with A, G, L, S, T, M, or V; N13 replaced with Q; Y14 replaced with F, or W; Y15 replaced with F, or W; T16 replaced with A, G, I, L, S, M, or V; S17 replaced with A, G, I, L, T, M, or V; E18 replaced with D; K22 replaced with H, or R; I23 replaced with A, G, L, S, T, M, or V; N24 replaced with Q; V25 replaced with A, G, I, L, S, T, or M; K26 replaced with H, or R; Q27 replaced with N; I28 replaced with A, G, L, S, T, M, or V; A29 replaced with G, I, L, S, T, M, or V; A30 replaced with G, I, L, S, T, M, or V; R31 replaced with H, or K; L32 replaced with A, G, I, S, T, M, or V; L33 replaced with A, G, I, S, T, M, or V; L36 replaced with A, G, I, S, T, M, or V; Y37 replaced with F, or W; S38 replaced with A, G, I, L, T, M, or V; L39 replaced with A, G, I, S, T, M, or V; V40 replaced with A, G, I, L, S, T, or M; F41 replaced with W, or Y; I42 replaced with A, G, L, S, T, M, or V; F43 replaced with W, or Y; G44 replaced with A, I, L, S, T, M, or V; F45 replaced with W, or Y; V46 replaced with A, G, I, L, S, T, or M; G47 replaced with A, I, L, S, T, M, or V; N48 replaced with Q; M49 replaced with A, G,

I, L, S, T, or V; L50 replaced with A, G, I, S, T, M, or V; V51 replaced with A, G, I, L, S, T, or M; I52 replaced with A, G, L, S, T, M, or V; L53 replaced with A, G, I, S, T, M, or V; I54 replaced with A, G, L, S, T, M, or V; L55 replaced with A, G, I, S, T, M, or V; I56 replaced with A, G, L, S, T, M, or V; N57 replaced with Q; Q59 replaced with N; R60 replaced with H, or K; L61 replaced with A, G, I, S, T, M, or V; E62 replaced with D; S63 replaced with A, G, I, L, T, M, or V; M64 replaced with A, G, I, L, S, T, or V; T65 replaced with A, G, I, L, S, M, or V; D66 replaced with E; I67 replaced with A, G, L, S, T, M, or V; Y68 replaced with F, or W; L69 replaced with A, G, I, S, T, M, or V; L70 replaced with A, G, I, S, T, M, or V; N71 replaced with Q; L72 replaced with A, G, I, S, T, M, or V; A73 replaced with G, I, L, S, T, M, or V; I74 replaced with A, G, L, S, T, M, or V; S75 replaced with A, G, I, L, T, M, or V; D76 replaced with E; L77 replaced with A, G, I, S, T, M, or V; F78 replaced with W, or Y; F79 replaced with W, or Y; L80 replaced with A, G, I, S, T, M, or V; L81 replaced with A, G, I, S, T, M, or V; T82 replaced with A, G, I, L, S, M, or V; V83 replaced with A, G, I, L, S, T, or M; F85 replaced with W, or Y; W86 replaced with F, or Y; A87 replaced with G, I, L, S, T, M, or V; H88 replaced with K, or R; Y89 replaced with F, or W; A90 replaced with G, I, L, S, T, M, or V; A91 replaced with G, I, L, S, T, M, or V; A92 replaced with G, I, L, S, T, M, or V; Q93 replaced with N; W94 replaced with F, or Y; D95 replaced with E; F96 replaced with W, or Y; G97 replaced with A, I, L, S, T, M, or V; N98 replaced with Q; T99 replaced with A, G, I, L, S, M, or V; M100 replaced with A, G, I, L, S, T, or V; Q102 replaced with N; L103 replaced with A, G, I, S, T, M, or V; L104 replaced with A, G, I, S, T, M, or V; T105 replaced with A, G, I, L, S, M, or V; G106 replaced with A, I, L, S, T, M, or V; L107 replaced with A, G, I, S, T, M, or V; Y108 replaced with F, or W; F109 replaced with W, or Y; I110 replaced with A, G, L, S, T, M, or V; G111 replaced with A, I, L, S, T, M, or V; F112 replaced with W, or Y; F113 replaced with W, or Y; S114 replaced with A, G, I, L, T, M, or V; G115 replaced with A, I, L, S, T, M, or V; I116 replaced with A, G, L, S, T, M, or V; F117 replaced with W, or Y; F118 replaced with W, or Y; I119 replaced with A, G, L, S, T, M, or V; I120 replaced with A, G, L, S, T, M, or V; L121 replaced with A, G, I, S, T, M, or V; L122 replaced with A, G, I, S, T, M, or V; T123 replaced with A, G, I, L, S, M, or V; I124 replaced with A, G, L, S, T, M, or V; D125 replaced with E; R126 replaced with H, or K; Y127 replaced with F, or W; L128 replaced with A, G, I, S, T, M, or V; A129 replaced with G, I, L, S, T, M, or V; I130 replaced with A, G, L, S, T, M, or V; V131 replaced with A, G, I, L, S, T, or M;

H132 replaced with K, or R; A133 replaced with G, I, L, S, T, M, or V; V134 replaced with A, G, I, L, S, T, or M; F135 replaced with W, or Y; A136 replaced with G, I, L, S, T, M, or V; L137 replaced with A, G, I, S, T, M, or V; K138 replaced with H, or R; A139 replaced with G, I, L, S, T, M, or V; R140 replaced with H, or K; T141 replaced with A, G, I, L, S, M, or V; V142 replaced with A, G, I, L, S, T, or M; T143 replaced with A, G, I, L, S, M, or V; F144 replaced with W, or Y; G145 replaced with A, I, L, S, T, M, or V; V146 replaced with A, G, I, L, S, T, or M; V147 replaced with A, G, I, L, S, T, or M; T148 replaced with A, G, I, L, S, M, or V; S149 replaced with A, G, I, L, T, M, or V; V150 replaced with A, G, I, L, S, T, or M; I151 replaced with A, G, L, S, T, M, or V; T152 replaced with A, G, I, L, S, M, or V; W153 replaced with F, or Y; V154 replaced with A, G, I, L, S, T, or M; V155 replaced with A, G, I, L, S, T, or M; A156 replaced with G, I, L, S, T, M, or V; V157 replaced with A, G, I, L, S, T, or M; F158 replaced with W, or Y; A159 replaced with G, I, L, S, T, M, or V; S160 replaced with A, G, I, L, T, M, or V; L161 replaced with A, G, I, S, T, M, or V; G163 replaced with A, I, L, S, T, M, or V; I164 replaced with A, G, L, S, T, M, or V; I165 replaced with A, G, L, S, T, M, or V; F166 replaced with W, or Y; T167 replaced with A, G, I, L, S, M, or V; R168 replaced with H, or K; S169 replaced with A, G, I, L, T, M, or V; Q170 replaced with N; K171 replaced with H, or R; E172 replaced with D; G173 replaced with A, I, L, S, T, M, or V; L174 replaced with A, G, I, S, T, M, or V; H175 replaced with K, or R; Y176 replaced with F, or W; T177 replaced with A, G, I, L, S, M, or V; S179 replaced with A, G, I, L, T, M, or V; S180 replaced with A, G, I, L, T, M, or V; H181 replaced with K, or R; F182 replaced with W, or Y; Y184 replaced with F, or W; S185 replaced with A, G, I, L, T, M, or V; Q186 replaced with N; Y187 replaced with F, or W; Q188 replaced with N; F189 replaced with W, or Y; W190 replaced with F, or Y; K191 replaced with H, or R; N192 replaced with Q; F193 replaced with W, or Y; Q194 replaced with N; T195 replaced with A, G, I, L, S, M, or V; L196 replaced with A, G, I, S, T, M, or V; K197 replaced with H, or R; I198 replaced with A, G, L, S, T, M, or V; V199 replaced with A, G, I, L, S, T, or M; I200 replaced with A, G, L, S, T, M, or V; L201 replaced with A, G, I, S, T, M, or V; G202 replaced with A, I, L, S, T, M, or V; L203 replaced with A, G, I, S, T, M, or V; V204 replaced with A, G, I, L, S, T, or M; L205 replaced with A, G, I, S, T, M, or V; L207 replaced with A, G, I, S, T, M, or V; L208 replaced with A, G, I, S, T, M, or V; V209 replaced with A, G, I, L, S, T, or M; M210 replaced with A, G, I, L, S, T, or V; V211 replaced with A, G, I, L, S, T, or M; I212 replaced with A, G, L, S, T, M, or V;

Y214 replaced with F, or W; S215 replaced with A, G, I, L, T, M, or V; G216 replaced with A, I, L, S, T, M, or V; I217 replaced with A, G, L, S, T, M, or V; L218 replaced with A, G, I, S, T, M, or V; K219 replaced with H, or R; T220 replaced with A, G, I, L, S, M, or V; L221 replaced with A, G, I, S, T, M, or V; L222 replaced with A, G, I, S, T, M, or V; R223 replaced with H, or K; R225 replaced with H, or K; N226 replaced with Q; E227 replaced with D; K228 replaced with H, or R; K229 replaced with H, or R; R230 replaced with H, or K; H231 replaced with K, or R; R232 replaced with H, or K; A233 replaced with G, I, L, S, T, M, or V; V234 replaced with A, G, I, L, S, T, or M; R235 replaced with H, or K; L236 replaced with A, G, I, S, T, M, or V; I237 replaced with A, G, L, S, T, M, or V; F238 replaced with W, or Y; T239 replaced with A, G, I, L, S, M, or V; I240 replaced with A, G, L, S, T, M, or V; M241 replaced with A, G, I, L, S, T, or V; I242 replaced with A, G, L, S, T, M, or V; V243 replaced with A, G, I, L, S, T, or M; Y244 replaced with F, or W; F245 replaced with W, or Y; L246 replaced with A, G, I, S, T, M, or V; F247 replaced with W, or Y; W248 replaced with F, or Y; A249 replaced with G, I, L, S, T, M, or V; Y251 replaced with F, or W; N252 replaced with Q; I253 replaced with A, G, L, S, T, M, or V; V254 replaced with A, G, I, L, S, T, or M; L255 replaced with A, G, I, S, T, M, or V; L256 replaced with A, G, I, S, T, M, or V; L257 replaced with A, G, I, S, T, M, or V; N258 replaced with Q; T259 replaced with A, G, I, L, S, M, or V; F260 replaced with W, or Y; Q261 replaced with N; E262 replaced with D; F263 replaced with W, or Y; F264 replaced with W, or Y; G265 replaced with A, I, L, S, T, M, or V; L266 replaced with A, G, I, S, T, M, or V; N267 replaced with Q; N268 replaced with Q; S270 replaced with A, G, I, L, T, M, or V; S271 replaced with A, G, I, L, T, M, or V; S272 replaced with A, G, I, L, T, M, or V; N273 replaced with Q; R274 replaced with H, or K; L275 replaced with A, G, I, S, T, M, or V; D276 replaced with E; Q277 replaced with N; A278 replaced with G, I, L, S, T, M, or V; M279 replaced with A, G, I, L, S, T, or V; Q280 replaced with N; V281 replaced with A, G, I, L, S, T, or M; T282 replaced with A, G, I, L, S, M, or V; E283 replaced with D; T284 replaced with A, G, I, L, S, M, or V; L285 replaced with A, G, I, S, T, M, or V; G286 replaced with A, I, L, S, T, M, or V; M287 replaced with A, G, I, L, S, T, or V; T288 replaced with A, G, I, L, S, M, or V; H289 replaced with K, or R; I292 replaced with A, G, L, S, T, M, or V; N293 replaced with Q; I295 replaced with A, G, L, S, T, M, or V; I296 replaced with A, G, L, S, T, M, or V; Y297 replaced with F, or W; A298 replaced with G, I, L, S, T, M, or V; F299 replaced with W, or Y; V300 replaced with A, G, I, L, S, T, or M; G301 replaced with A, I, L, S,

T, M, or V; E302 replaced with D; K303 replaced with H, or R; F304 replaced with W, or Y; R305 replaced with H, or K; N306 replaced with Q; Y307 replaced with F, or W; L308 replaced with A, G, I, S, T, M, or V; L309 replaced with A, G, I, S, T, M, or V; V310 replaced with A, G, I, L, S, T, or M; F311 replaced with W, or Y; F312 replaced with W, or Y; Q313 replaced with N; K314 replaced with H, or R; H315 replaced with K, or R; I316 replaced with A, G, L, S, T, M, or V; A317 replaced with G, I, L, S, T, M, or V; K318 replaced with H, or R; R319 replaced with H, or K; F320 replaced with W, or Y; K322 replaced with H, or R; S325 replaced with A, G, I, L, T, M, or V; I326 replaced with A, G, L, S, T, M, or V; F327 replaced with W, or Y; Q328 replaced with N; Q329 replaced with N; E330 replaced with D; A331 replaced with G, I, L, S, T, M, or V; E333 replaced with D; R334 replaced with H, or K; A335 replaced with G, I, L, S, T, M, or V; S336 replaced with A, G, I, L, T, M, or V; S337 replaced with A, G, I, L, T, M, or V; V338 replaced with A, G, I, L, S, T, or M; Y339 replaced with F, or W; T340 replaced with A, G, I, L, S, M, or V; R341 replaced with H, or K; S342 replaced with A, G, I, L, T, M, or V; T343 replaced with A, G, I, L, S, M, or V; G344 replaced with A, I, L, S, T, M, or V; E345 replaced with D; Q346 replaced with N; E347 replaced with D; I348 replaced with A, G, L, S, T, M, or V; S349 replaced with A, G, I, L, T, M, or V; V350 replaced with A, G, I, L, S, T, or M; G351 replaced with A, I, L, S, T, M, or V; and/or L352 replaced with A, G, I, S, T, M, or V.

Preferred conservative mutations of G-protein Chemokine Receptor (CCR5) as encoded by the deposited HDGNR10 clone (SEQ ID NO:22) include: M1 replaced with A, G, I, L, S, T, or V; D2 replaced with E; Y3 replaced with F, or W; Q4 replaced with N; V5 replaced with A, G, I, L, S, T, or M; S6 replaced with A, G, I, L, T, M, or V; S7 replaced with A, G, I, L, T, M, or V; I9 replaced with A, G, L, S, T, M, or V; Y10 replaced with F, or W; D11 replaced with E; I12 replaced with A, G, L, S, T, M, or V; N13 replaced with Q; Y14 replaced with F, or W; Y15 replaced with F, or W; T16 replaced with A, G, I, L, S, M, or V; S17 replaced with A, G, I, L, T, M, or V; E18 replaced with D; Q21 replaced with N; K22 replaced with H, or R; I23 replaced with A, G, L, S, T, M, or V; N24 replaced with Q; V25 replaced with A, G, I, L, S, T, or M; K26 replaced with H, or R; Q27 replaced with N; I28 replaced with A, G, L, S, T, M, or V; A29 replaced with G, I, L, S, T, M, or V; A30 replaced with G, I, L, S, T, M, or V; R31 replaced with H, or K; L32 replaced with A, G, I, S, T, M, or V; L33 replaced with A, G, I, S, T, M, or V; L36 replaced with A, G, I, S, T, M, or V; Y37 replaced with F, or W; S38 replaced with A, G, I,

L, T, M, or V; L39 replaced with A, G, I, S, T, M, or V; V40 replaced with A, G, I, L, S, T, or M; F41 replaced with W, or Y; I42 replaced with A, G, L, S, T, M, or V; F43 replaced with W, or Y; G44 replaced with A, I, L, S, T, M, or V; F45 replaced with W, or Y; V46 replaced with A, G, I, L, S, T, or M; G47 replaced with A, I, L, S, T, M, or V; N48 replaced with Q; M49 replaced with A, G, I, L, S, T, or V; L50 replaced with A, G, I, S, T, M, or V; V51 replaced with A, G, I, L, S, T, or M; I52 replaced with A, G, L, S, T, M, or V; L53 replaced with A, G, I, S, T, M, or V; I54 replaced with A, G, L, S, T, M, or V; L55 replaced with A, G, I, S, T, M, or V; I56 replaced with A, G, L, S, T, M, or V; N57 replaced with Q; K59 replaced with H, or R; R60 replaced with H, or K; L61 replaced with A, G, I, S, T, M, or V; K62 replaced with H, or R; S63 replaced with A, G, I, L, T, M, or V; M64 replaced with A, G, I, L, S, T, or V; T65 replaced with A, G, I, L, S, M, or V; D66 replaced with E; I67 replaced with A, G, L, S, T, M, or V; Y68 replaced with F, or W; L69 replaced with A, G, I, S, T, M, or V; L70 replaced with A, G, I, S, T, M, or V; N71 replaced with Q; L72 replaced with A, G, I, S, T, M, or V; A73 replaced with G, I, L, S, T, M, or V; I74 replaced with A, G, L, S, T, M, or V; S75 replaced with A, G, I, L, T, M, or V; D76 replaced with E; L77 replaced with A, G, I, S, T, M, or V; F78 replaced with W, or Y; F79 replaced with W, or Y; L80 replaced with A, G, I, S, T, M, or V; L81 replaced with A, G, I, S, T, M, or V; T82 replaced with A, G, I, L, S, M, or V; V83 replaced with A, G, I, L, S, T, or M; F85 replaced with W, or Y; W86 replaced with F, or Y; A87 replaced with G, I, L, S, T, M, or V; H88 replaced with K, or R; Y89 replaced with F, or W; A90 replaced with G, I, L, S, T, M, or V; A91 replaced with G, I, L, S, T, M, or V; A92 replaced with G, I, L, S, T, M, or V; Q93 replaced with N; W94 replaced with F, or Y; D95 replaced with E; F96 replaced with W, or Y; G97 replaced with A, I, L, S, T, M, or V; N98 replaced with Q; T99 replaced with A, G, I, L, S, M, or V; M100 replaced with A, G, I, L, S, T, or V; Q102 replaced with N; L103 replaced with A, G, I, S, T, M, or V; L104 replaced with A, G, I, S, T, M, or V; T105 replaced with A, G, I, L, S, M, or V; G106 replaced with A, I, L, S, T, M, or V; L107 replaced with A, G, I, S, T, M, or V; Y108 replaced with F, or W; F109 replaced with W, or Y; I110 replaced with A, G, L, S, T, M, or V; G111 replaced with A, I, L, S, T, M, or V; F112 replaced with W, or Y; F113 replaced with W, or Y; S114 replaced with A, G, I, L, T, M, or V; G115 replaced with A, I, L, S, T, M, or V; I116 replaced with A, G, L, S, T, M, or V; F117 replaced with W, or Y; F118 replaced with W, or Y; I119 replaced with A, G, L, S, T, M, or V; I120 replaced with A, G, L, S, T, M, or V; L121 replaced with A, G, I, S, T, M, or V; L122 replaced

with A, G, I, S, T, M, or V; T123 replaced with A, G, I, L, S, M, or V; I124 replaced with A, G, L, S, T, M, or V; D125 replaced with E; R126 replaced with H, or K; Y127 replaced with F, or W; L128 replaced with A, G, I, S, T, M, or V; A129 replaced with G, I, L, S, T, M, or V; V130 replaced with A, G, I, L, S, T, or M; V131 replaced with A, G, I, L, S, T, or M; H132 replaced with K, or R; A133 replaced with G, I, L, S, T, M, or V; V134 replaced with A, G, I, L, S, T, or M; F135 replaced with W, or Y; A136 replaced with G, I, L, S, T, M, or V; L137 replaced with A, G, I, S, T, M, or V; K138 replaced with H, or R; A139 replaced with G, I, L, S, T, M, or V; R140 replaced with H, or K; T141 replaced with A, G, I, L, S, M, or V; V142 replaced with A, G, I, L, S, T, or M; T143 replaced with A, G, I, L, S, M, or V; F144 replaced with W, or Y; G145 replaced with A, I, L, S, T, M, or V; V146 replaced with A, G, I, L, S, T, or M; V147 replaced with A, G, I, L, S, T, or M; T148 replaced with A, G, I, L, S, M, or V; S149 replaced with A, G, I, L, T, M, or V; V150 replaced with A, G, I, L, S, T, or M; I151 replaced with A, G, L, S, T, M, or V; T152 replaced with A, G, I, L, S, M, or V; W153 replaced with F, or Y; V154 replaced with A, G, I, L, S, T, or M; V155 replaced with A, G, I, L, S, T, or M; A156 replaced with G, I, L, S, T, M, or V; V157 replaced with A, G, I, L, S, T, or M; F158 replaced with W, or Y; A159 replaced with G, I, L, S, T, M, or V; S160 replaced with A, G, I, L, T, M, or V; L161 replaced with A, G, I, S, T, M, or V; G163 replaced with A, I, L, S, T, M, or V; I164 replaced with A, G, L, S, T, M, or V; I165 replaced with A, G, L, S, T, M, or V; F166 replaced with W, or Y; T167 replaced with A, G, I, L, S, M, or V; R168 replaced with H, or K; S169 replaced with A, G, I, L, T, M, or V; Q170 replaced with N; K171 replaced with H, or R; E172 replaced with D; G173 replaced with A, I, L, S, T, M, or V; L174 replaced with A, G, I, S, T, M, or V; H175 replaced with K, or R; Y176 replaced with F, or W; T177 replaced with A, G, I, L, S, M, or V; S179 replaced with A, G, I, L, T, M, or V; S180 replaced with A, G, I, L, T, M, or V; H181 replaced with K, or R; F182 replaced with W, or Y; Y184 replaced with F, or W; S185 replaced with A, G, I, L, T, M, or V; Q186 replaced with N; Y187 replaced with F, or W; Q188 replaced with N; F189 replaced with W, or Y; W190 replaced with F, or Y; K191 replaced with H, or R; N192 replaced with Q; F193 replaced with W, or Y; Q194 replaced with N; T195 replaced with A, G, I, L, S, M, or V; L196 replaced with A, G, I, S, T, M, or V; K197 replaced with H, or R; I198 replaced with A, G, L, S, T, M, or V; V199 replaced with A, G, I, L, S, T, or M; I200 replaced with A, G, L, S, T, M, or V; L201 replaced with A, G, I, S, T, M, or V; G202 replaced with A, I, L, S, T, M, or V; L203 replaced with A, G, I, S, T, M, or V; V204

replaced with A, G, I, L, S, T, or M; L205 replaced with A, G, I, S, T, M, or V; L207 replaced with A, G, I, S, T, M, or V; L208 replaced with A, G, I, S, T, M, or V; V209 replaced with A, G, I, L, S, T, or M; M210 replaced with A, G, I, L, S, T, or V; V211 replaced with A, G, I, L, S, T, or M; I212 replaced with A, G, L, S, T, M, or V; Y214 replaced with F, or W; S215 replaced with A, G, I, L, T, M, or V; G216 replaced with A, I, L, S, T, M, or V; I217 replaced with A, G, L, S, T, M, or V; L218 replaced with A, G, I, S, T, M, or V; K219 replaced with H, or R; T220 replaced with A, G, I, L, S, M, or V; L221 replaced with A, G, I, S, T, M, or V; L222 replaced with A, G, I, S, T, M, or V; R223 replaced with H, or K; R225 replaced with H, or K; N226 replaced with Q; E227 replaced with D; K228 replaced with H, or R; K229 replaced with H, or R; R230 replaced with H, or K; H231 replaced with K, or R; R232 replaced with H, or K; A233 replaced with G, I, L, S, T, M, or V; V234 replaced with A, G, I, L, S, T, or M; R235 replaced with H, or K; L236 replaced with A, G, I, S, T, M, or V; I237 replaced with A, G, L, S, T, M, or V; F238 replaced with W, or Y; T239 replaced with A, G, I, L, S, M, or V; I240 replaced with A, G, L, S, T, M, or V; M241 replaced with A, G, I, L, S, T, or V; I242 replaced with A, G, L, S, T, M, or V; V243 replaced with A, G, I, L, S, T, or M; Y244 replaced with F, or W; F245 replaced with W, or Y; L246 replaced with A, G, I, S, T, M, or V; F247 replaced with W, or Y; W248 replaced with F, or Y; A249 replaced with G, I, L, S, T, M, or V; Y251 replaced with F, or W; N252 replaced with Q; I253 replaced with A, G, L, S, T, M, or V; V254 replaced with A, G, I, L, S, T, or M; L255 replaced with A, G, I, S, T, M, or V; L256 replaced with A, G, I, S, T, M, or V; L257 replaced with A, G, I, S, T, M, or V; N258 replaced with Q; T259 replaced with A, G, I, L, S, M, or V; F260 replaced with W, or Y; Q261 replaced with N; E262 replaced with D; F263 replaced with W, or Y; F264 replaced with W, or Y; G265 replaced with A, I, L, S, T, M, or V; L266 replaced with A, G, I, S, T, M, or V; N267 replaced with Q; N268 replaced with Q; S270 replaced with A, G, I, L, T, M, or V; S271 replaced with A, G, I, L, T, M, or V; S272 replaced with A, G, I, L, T, M, or V; N273 replaced with Q; R274 replaced with H, or K; L275 replaced with A, G, I, S, T, M, or V; D276 replaced with E; Q277 replaced with N; A278 replaced with G, I, L, S, T, M, or V; M279 replaced with A, G, I, L, S, T, or V; Q280 replaced with N; V281 replaced with A, G, I, L, S, T, or M; T282 replaced with A, G, I, L, S, M, or V; E283 replaced with D; T284 replaced with A, G, I, L, S, M, or V; L285 replaced with A, G, I, S, T, M, or V; G286 replaced with A, I, L, S, T, M, or V; M287 replaced with A, G, I, L, S, T, or V; T288 replaced with A, G, I, L, S, M, or V; H289 replaced with K, or R; I292

replaced with A, G, L, S, T, M, or V; N293 replaced with Q; I295 replaced with A, G, L, S, T, M, or V; I296 replaced with A, G, L, S, T, M, or V; Y297 replaced with F, or W; A298 replaced with G, I, L, S, T, M, or V; F299 replaced with W, or Y; V300 replaced with A, G, I, L, S, T, or M; G301 replaced with A, I, L, S, T, M, or V; E302 replaced with D; K303 replaced with H, or R; F304 replaced with W, or Y; R305 replaced with H, or K; N306 replaced with Q; Y307 replaced with F, or W; L308 replaced with A, G, I, S, T, M, or V; L309 replaced with A, G, I, S, T, M, or V; V310 replaced with A, G, I, L, S, T, or M; F311 replaced with W, or Y; F312 replaced with W, or Y; Q313 replaced with N; K314 replaced with H, or R; H315 replaced with K, or R; I316 replaced with A, G, L, S, T, M, or V; A317 replaced with G, I, L, S, T, M, or V; K318 replaced with H, or R; R319 replaced with H, or K; F320 replaced with W, or Y; K322 replaced with H, or R; S325 replaced with A, G, I, L, T, M, or V; I326 replaced with A, G, L, S, T, M, or V; F327 replaced with W, or Y; Q328 replaced with N; Q329 replaced with N; E330 replaced with D; A331 replaced with G, I, L, S, T, M, or V; E333 replaced with D; R334 replaced with H, or K; A335 replaced with G, I, L, S, T, M, or V; S336 replaced with A, G, I, L, T, M, or V; S337 replaced with A, G, I, L, T, M, or V; V338 replaced with A, G, I, L, S, T, or M; Y339 replaced with F, or W; T340 replaced with A, G, I, L, S, M, or V; R341 replaced with H, or K; S342 replaced with A, G, I, L, T, M, or V; T343 replaced with A, G, I, L, S, M, or V; E344 replaced with D; E345 replaced with D; Q346 replaced with N; E347 replaced with D; I348 replaced with A, G, L, S, T, M, or V; S349 replaced with A, G, I, L, T, M, or V; V350 replaced with A, G, I, L, S, T, or M; G351 replaced with A, I, L, S, T, M, or V; and/or L352 replaced with A, G, I, S, T, M, or V.

The resulting constructs can be routinely screened for activities or functions described throughout the specification and known in the art. Preferably, the resulting constructs have an increased and/or a decreased G-protein Chemokine Receptor (CCR5) activity or function, while the remaining G-protein Chemokine Receptor (CCR5) activities or functions are maintained. More preferably, the resulting constructs have more than one increased and/or decreased G-protein Chemokine Receptor (CCR5) activity or function, while the remaining G-protein Chemokine Receptor (CCR5) activities or functions are maintained.

Besides conservative amino acid substitution, variants of G-protein Chemokine Receptor (CCR5) include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded

by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, G-protein Chemokine Receptor (CCR5) polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

For example, preferred non-conservative substitutions of G-protein Chemokine Receptor (CCR5) (SEQ ID NO:2) include: M1 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D2 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y3 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q4 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V5 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S6 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S7 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P8 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I9 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y10 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D11 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I12 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N13 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y14 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Y15 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T16 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S17 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E18 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P19 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; C20 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; P21 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; K22 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I23 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N24 replaced with D, E, H, K, R, A,

G, I, L, S, T, M, V, F, W, Y, P, or C; V25 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K26 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q27 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I28 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A29 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A30 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R31 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L32 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L33 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P34 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P35 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L36 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y37 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S38 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L39 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V40 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F41 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I42 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F43 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G44 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F45 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V46 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G47 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N48 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; M49 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L50 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V51 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I52 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L53 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I54 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L55 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I56 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N57 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; C58 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Q59 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R60 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L61 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E62 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S63 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M64 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T65 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D66 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I67 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y68 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L69 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L70 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N71 replaced

with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L72 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A73 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I74 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S75 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D76 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L77 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F78 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F79 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L80 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L81 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T82 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V83 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P84 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; F85 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; W86 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A87 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H88 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y89 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A90 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A91 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A92 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q93 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; W94 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D95 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F96 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G97 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N98 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T99 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M100 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C101 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Q102 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L103 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L104 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T105 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G106 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L107 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y108 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F109 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I110 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G111 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F112 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F113 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S114 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G115 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I116 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C;

C;F117 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F118 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I119 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I120 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L121 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L122 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T123 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I124 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D125 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R126 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y127 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L128 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A129 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I130 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V131 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H132 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A133 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V134 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F135 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A136 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L137 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K138 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A139 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R140 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T141 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V142 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T143 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F144 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G145 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V146 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V147 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T148 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S149 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V150 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I151 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T152 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W153 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V154 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V155 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A156 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V157 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F158 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A159 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S160 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L161 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P162 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G163 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I164 replaced with D,

E, H, K, R, N, Q, F, W, Y, P, or C; I165 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F166 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T167 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R168 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S169 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q170 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K171 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E172 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G173 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L174 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H175 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y176 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T177 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C178 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; S179 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S180 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H181 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F182 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; P183 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Y184 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S185 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q186 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y187 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q188 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; F189 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; W190 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K191 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N192 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; F193 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q194 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T195 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L196 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K197 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I198 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V199 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I200 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L201 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G202 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L203 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V204 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L205 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P206 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L207 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L208 replaced with D, E, H, K, R, N, Q, F, W,

Y, P, or C; V209 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M210 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V211 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I212 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C213 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Y214 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S215 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G216 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I217 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L218 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K219 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T220 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L221 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L222 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R223 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C224 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; R225 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N226 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E227 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K228 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K229 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R230 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H231 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R232 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A233 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V234 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R235 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L236 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I237 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F238 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T239 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I240 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M241 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I242 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V243 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y244 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F245 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L246 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F247 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; W248 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A249 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P250 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Y251 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N252 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I253 replaced with D, E, H, K, R, N, Q, F,

W, Y, P, or C; V254 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L255 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L256 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L257 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N258 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T259 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F260 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q261 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E262 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F263 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F264 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G265 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L266 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N267 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N268 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; C269 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; S270 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S271 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S272 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N273 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R274 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L275 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D276 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q277 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A278 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M279 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q280 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V281 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T282 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E283 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T284 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L285 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G286 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M287 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T288 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H289 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C290 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; C291 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; I292 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N293 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P294 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I295 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I296 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y297 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or

C; A298 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F299 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V300 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G301 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E302 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K303 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F304 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R305 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N306 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y307 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L308 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L309 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V310 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F311 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F312 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q313 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K314 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H315 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I316 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A317 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K318 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R319 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F320 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; C321 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; K322 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C323 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; C324 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; S325 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I326 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F327 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q328 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q329 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E330 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A331 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P332 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E333 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R334 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A335 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S336 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S337 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V338 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y339 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T340 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R341

replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S342 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T343 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G344 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E345 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q346 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E347 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I348 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S349 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V350 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G351 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; and/or L352 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C.

Addition preferred non-conservative substitutions of G-protein Chemokine Receptor (CCR5) as encoded by the HDG NR10 deposited clone (SEQ ID NO:22) include: M1 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D2 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y3 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q4 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V5 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S6 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S7 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P8 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I9 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y10 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D11 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I12 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N13 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y14 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Y15 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T16 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S17 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E18 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; P19 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; C20 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Q21 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K22 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I23 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N24 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V25 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K26 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q27 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I28 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A29 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A30 replaced with D, E, H, K, R, N,

Q, F, W, Y, P, or C; R31 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L32 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L33 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P34 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; P35 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L36 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y37 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S38 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L39 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V40 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F41 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I42 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F43 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G44 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F45 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V46 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G47 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N48 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; M49 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L50 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V51 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I52 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L53 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I54 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L55 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I56 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N57 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; C58 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; K59 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R60 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L61 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K62 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S63 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M64 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T65 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D66 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I67 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y68 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L69 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L70 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N71 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L72 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A73 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I74 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S75 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D76 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L77 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C;

or C; F78 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F79 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L80 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L81 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T82 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V83 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P84 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; F85 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; W86 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A87 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H88 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y89 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A90 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A91 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A92 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q93 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; W94 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; D95 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F96 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G97 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N98 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T99 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M100 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C101 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Q102 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; L103 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L104 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T105 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G106 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L107 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y108 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F109 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I110 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G111 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F112 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F113 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S114 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G115 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I116 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F117 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F118 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; I119 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I120 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L121 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L122 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T123 replaced with D, E, H, K, R, N,

Q, F, W, Y, P, or C; I124 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D125 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R126 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y127 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L128 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A129 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V130 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V131 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H132 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A133 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V134 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F135 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A136 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L137 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K138 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A139 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R140 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T141 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V142 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T143 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F144 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G145 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V146 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V147 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T148 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S149 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V150 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I151 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T152 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; W153 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V154 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V155 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A156 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V157 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F158 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A159 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S160 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L161 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P162 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; G163 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I164 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I165 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F166 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T167 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R168 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S169 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q170 replaced with D, E, H, K, R, A, G, I, L,

S, T, M, V, F, W, Y, P, or C; K171 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E172 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; G173 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L174 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H175 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Y176 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T177 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C178 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; S179 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S180 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H181 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F182 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; P183 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Y184 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; S185 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q186 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y187 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q188 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; F189 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; W190 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; K191 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N192 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; F193 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q194 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T195 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L196 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K197 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I198 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V199 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I200 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L201 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G202 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L203 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V204 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L205 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P206 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; L207 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L208 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V209 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M210 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V211 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I212 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; C213 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; Y214 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C;

S215 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G216 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I217 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L218 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K219 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T220 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L221 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L222 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R223 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C224 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; R225 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N226 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E227 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K228 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K229 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R230 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H231 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R232 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A233 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V234 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R235 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L236 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I237 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F238 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T239 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I240 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M241 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I242 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V243 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y244 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F245 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L246 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F247 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; W248 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A249 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P250 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; Y251 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; N252 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; I253 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V254 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L255 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L256 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L257 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N258 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; T259 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F260

replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q261 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E262 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F263 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F264 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; G265 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L266 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N267 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; N268 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; C269 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; S270 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S271 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S272 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N273 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; R274 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; L275 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; D276 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q277 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; A278 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M279 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Q280 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; V281 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T282 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E283 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; T284 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L285 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G286 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; M287 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T288 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; H289 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C290 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; C291 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; I292 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; N293 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; P294 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; I295 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I296 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y297 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; A298 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F299 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; V300 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G301 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E302 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; K303 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F304

replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; R305 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; N306 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Y307 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; L308 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; L309 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V310 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F311 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; F312 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q313 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; K314 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; H315 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I316 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; A317 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; K318 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R319 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; F320 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; C321 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; K322 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; C323 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; C324 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or P; S325 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; I326 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; F327 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; Q328 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; Q329 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E330 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A331 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; P332 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, or C; E333 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; R334 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; A335 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S336 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S337 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V338 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; Y339 replaced with D, E, H, K, R, N, Q, A, G, I, L, S, T, M, V, P, or C; T340 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; R341 replaced with D, E, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; S342 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; T343 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; E344 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; E345 replaced with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; Q346 replaced with D, E, H, K, R, A, G, I, L, S, T, M, V, F, W, Y, P, or C; E347 replaced

with H, K, R, A, G, I, L, S, T, M, V, N, Q, F, W, Y, P, or C; I348 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; S349 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; V350 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; G351 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C; and/or L352 replaced with D, E, H, K, R, N, Q, F, W, Y, P, or C.

The resulting constructs can be routinely screened for activities or functions described throughout the specification and known in the art. Preferably, the resulting constructs have an increased and/or decreased G-protein Chemokine Receptor (CCR5) activity or function, while the remaining G-protein Chemokine Receptor (CCR5) activities or functions are maintained. More preferably, the resulting constructs have more than one increased and/or decreased G-protein Chemokine Receptor (CCR5) activity or function, while the remaining G-protein Chemokine Receptor (CCR5) activities or functions are maintained.

Additionally, more than one amino acid (e.g., 2, 3, 4, 5, 6, 7, 8, 9 and 10) can be replaced with the substituted amino acids as described above (either conservative or nonconservative). The substituted amino acids can occur in the full length, mature, or proprotein form of G-protein Chemokine Receptor (CCR5) protein, as well as the N- and C- terminal deletion mutants, having the general formula m-n, listed below.

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a G-protein Chemokine Receptor (CCR5) polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course, in order of ever-increasing preference, it is highly preferable for a polypeptide to have an amino acid sequence which comprises the amino acid sequence of a G-protein Chemokine Receptor (CCR5) polypeptide, which contains at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of FIG. 1 or that encoded by the deposited clone or fragments thereof (e.g., the mature form and/or other fragments described herein), is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the polynucleotides of the invention. In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence which: is a portion of that contained in a deposited clone, or encoding the polypeptide encoded by deposited clone; is a portion of that shown in SEQ ID NO:1 or the complementary strand thereto, or is a portion of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the HDGNR10 DNA sequence contained in a deposited clone or the nucleotide sequence shown in SEQ ID NO:1. In this context "about" includes the particularly recited value, a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500, 600, 1000 nucleotides) are preferred.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700, 701-750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, or 1401 to the end of SEQ ID NO:1, or the complementary strand thereto, or the HDGNR10 DNA contained in the deposited clone. In this context "about" includes the particularly recited ranges, and ranges larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to these nucleic acid molecules under stringent hybridization conditions or lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides. In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:2 or encoded by the HDGNR10 DNA contained in the deposited

clone. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, or 161 to the end of the coding region. Moreover, polypeptide fragments can be about 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, and ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind G-protein Chemokine Receptor (CCR5) ligand) may still be retained. For example, the ability of shortened G-protein Chemokine Receptor (CCR5) muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that an G-protein Chemokine Receptor (CCR5) mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six G-protein Chemokine Receptor (CCR5) amino acid residues may often evoke an immune response.

Preferred polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids,

Accordingly, polypeptide fragments include the secreted G-protein Chemokine Receptor (CCR5) protein as well as the mature form. Further preferred polypeptide fragments include the secreted G-protein Chemokine Receptor (CCR5) protein or the mature form having a continuous series of deleted residues from the amino or the carboxy

terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted G-protein Chemokine Receptor (CCR5) polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted G-protein Chemokine Receptor (CCR5) protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

Particularly, N-terminal deletions of the G-protein Chemokine Receptor (CCR5) polypeptide can be described by the general formula m-352, where m is an integer from 2 to 346, where m corresponds to the position of the amino acid residue identified in SEQ ID NO:2 or the polypeptide encoded by the deposited clone. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of N-terminal deletions of the polypeptide of the invention shown as SEQ ID NO:2 including polypeptides comprising the amino acid sequence of residues: D-2 to L-352; Y-3 to L-352; Q-4 to L-352; V-5 to L-352; S-6 to L-352; S-7 to L-352; P-8 to L-352; I-9 to L-352; Y-10 to L-352; D-11 to L-352; I-12 to L-352; N-13 to L-352; Y-14 to L-352; Y-15 to L-352; T-16 to L-352; S-17 to L-352; E-18 to L-352; P-19 to L-352; C-20 to L-352; P-21 to L-352; K-22 to L-352; I-23 to L-352; N-24 to L-352; V-25 to L-352; K-26 to L-352; Q-27 to L-352; I-28 to L-352; A-29 to L-352; A-30 to L-352; R-31 to L-352; L-32 to L-352; L-33 to L-352; P-34 to L-352; P-35 to L-352; L-36 to L-352; Y-37 to L-352; S-38 to L-352; L-39 to L-352; V-40 to L-352; F-41 to L-352; I-42 to L-352; F-43 to L-352; G-44 to L-352; F-45 to L-352; V-46 to L-352; G-47 to L-352; N-48 to L-352; M-49 to L-352; L-50 to L-352; V-51 to L-352; I-52 to L-352; L-53 to L-352; I-54 to L-352; L-55 to L-352; I-56 to L-352; N-57 to L-352; C-58 to L-352; Q-59 to L-352; R-60 to L-352; L-61 to L-352; E-62 to L-352; S-63 to L-352; M-64 to L-352; T-65 to L-352; D-66 to L-352; I-67 to L-352; Y-68 to L-352; L-69 to L-352; L-70 to L-352; N-71 to L-352; L-72 to L-352; A-73 to L-352; I-74 to L-352; S-75 to L-352; D-76 to L-352; L-77 to L-352; F-78 to L-352; F-79 to L-352; L-80 to L-352; L-81 to L-352; T-82 to L-352; V-83 to L-352; P-84 to L-352; F-85 to L-352; W-86 to L-352; A-87 to L-352; H-88 to L-352; Y-89 to L-352; A-90 to L-352; A-91 to L-352; A-92 to L-352; Q-93 to L-352; W-94 to L-352; D-95 to L-352; F-96 to L-352; G-97 to L-352; N-98 to L-352; T-99 to L-352; M-100 to L-352; C-101 to L-352; Q-102 to L-352; L-103 to L-352; L-104 to L-352; T-105 to L-352; G-106 to L-352; L-107 to L-352; Y-108 to L-352; F-109 to

L-352; I-110 to L-352; G-111 to L-352; F-112 to L-352; F-113 to L-352; S-114 to L-352; G-115 to L-352; I-116 to L-352; F-117 to L-352; F-118 to L-352; I-119 to L-352; I-120 to L-352; L-121 to L-352; L-122 to L-352; T-123 to L-352; I-124 to L-352; D-125 to L-352; R-126 to L-352; Y-127 to L-352; L-128 to L-352; A-129 to L-352; I-130 to L-352; V-131 to L-352; H-132 to L-352; A-133 to L-352; V-134 to L-352; F-135 to L-352; A-136 to L-352; L-137 to L-352; K-138 to L-352; A-139 to L-352; R-140 to L-352; T-141 to L-352; V-142 to L-352; T-143 to L-352; F-144 to L-352; G-145 to L-352; V-146 to L-352; V-147 to L-352; T-148 to L-352; S-149 to L-352; V-150 to L-352; I-151 to L-352; T-152 to L-352; W-153 to L-352; V-154 to L-352; V-155 to L-352; A-156 to L-352; V-157 to L-352; F-158 to L-352; A-159 to L-352; S-160 to L-352; L-161 to L-352; P-162 to L-352; G-163 to L-352; I-164 to L-352; I-165 to L-352; F-166 to L-352; T-167 to L-352; R-168 to L-352; S-169 to L-352; Q-170 to L-352; K-171 to L-352; E-172 to L-352; G-173 to L-352; L-174 to L-352; H-175 to L-352; Y-176 to L-352; T-177 to L-352; C-178 to L-352; S-179 to L-352; S-180 to L-352; H-181 to L-352; F-182 to L-352; P-183 to L-352; Y-184 to L-352; S-185 to L-352; Q-186 to L-352; Y-187 to L-352; Q-188 to L-352; F-189 to L-352; W-190 to L-352; K-191 to L-352; N-192 to L-352; F-193 to L-352; Q-194 to L-352; T-195 to L-352; L-196 to L-352; K-197 to L-352; I-198 to L-352; V-199 to L-352; I-200 to L-352; L-201 to L-352; G-202 to L-352; L-203 to L-352; V-204 to L-352; L-205 to L-352; P-206 to L-352; L-207 to L-352; L-208 to L-352; V-209 to L-352; M-210 to L-352; V-211 to L-352; I-212 to L-352; C-213 to L-352; Y-214 to L-352; S-215 to L-352; G-216 to L-352; I-217 to L-352; L-218 to L-352; K-219 to L-352; T-220 to L-352; L-221 to L-352; L-222 to L-352; R-223 to L-352; C-224 to L-352; R-225 to L-352; N-226 to L-352; E-227 to L-352; K-228 to L-352; K-229 to L-352; R-230 to L-352; H-231 to L-352; R-232 to L-352; A-233 to L-352; V-234 to L-352; R-235 to L-352; L-236 to L-352; I-237 to L-352; F-238 to L-352; T-239 to L-352; I-240 to L-352; M-241 to L-352; I-242 to L-352; V-243 to L-352; Y-244 to L-352; F-245 to L-352; L-246 to L-352; F-247 to L-352; W-248 to L-352; A-249 to L-352; P-250 to L-352; Y-251 to L-352; N-252 to L-352; I-253 to L-352; V-254 to L-352; L-255 to L-352; L-256 to L-352; L-257 to L-352; N-258 to L-352; T-259 to L-352; F-260 to L-352; Q-261 to L-352; E-262 to L-352; F-263 to L-352; F-264 to L-352; G-265 to L-352; L-266 to L-352; N-267 to L-352; N-268 to L-352; C-269 to L-352; S-270 to L-352; S-271 to L-352; S-272 to L-352; N-273 to L-352; R-274 to L-352; L-275 to L-352; D-276 to L-352; Q-277 to L-352; A-278 to L-352; M-279 to L-352; Q-280 to L-352; V-281 to L-352; T-282 to L-352; E-283 to L-352; T-284 to L-352; L-285 to L-352;

G-286 to L-352; M-287 to L-352; T-288 to L-352; H-289 to L-352; C-290 to L-352; C-291 to L-352; I-292 to L-352; N-293 to L-352; P-294 to L-352; I-295 to L-352; I-296 to L-352; Y-297 to L-352; A-298 to L-352; F-299 to L-352; V-300 to L-352; G-301 to L-352; E-302 to L-352; K-303 to L-352; F-304 to L-352; R-305 to L-352; N-306 to L-352; Y-307 to L-352; L-308 to L-352; L-309 to L-352; V-310 to L-352; F-311 to L-352; F-312 to L-352; Q-313 to L-352; K-314 to L-352; H-315 to L-352; I-316 to L-352; A-317 to L-352; K-318 to L-352; R-319 to L-352; F-320 to L-352; C-321 to L-352; K-322 to L-352; C-323 to L-352; C-324 to L-352; S-325 to L-352; I-326 to L-352; F-327 to L-352; Q-328 to L-352; Q-329 to L-352; E-330 to L-352; A-331 to L-352; P-332 to L-352; E-333 to L-352; R-334 to L-352; A-335 to L-352; S-336 to L-352; S-337 to L-352; V-338 to L-352; Y-339 to L-352; T-340 to L-352; R-341 to L-352; S-342 to L-352; T-343 to L-352; G-344 to L-352; E-345 to L-352; Q-346 to L-352; and/or E-347 to L-352 of SEQ ID NO:2. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In addition, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of N-terminal deletions of the polypeptide of the invention encoded by the deposited HDGNR10 clone (SEQ ID NO:22) including polypeptides comprising the amino acid sequence of residues: D-2 to L-352; Y-3 to L-352; Q-4 to L-352; V-5 to L-352; S-6 to L-352; S-7 to L-352; P-8 to L-352; I-9 to L-352; Y-10 to L-352; D-11 to L-352; I-12 to L-352; N-13 to L-352; Y-14 to L-352; Y-15 to L-352; T-16 to L-352; S-17 to L-352; E-18 to L-352; P-19 to L-352; C-20 to L-352; Q-21 to L-352; K-22 to L-352; I-23 to L-352; N-24 to L-352; V-25 to L-352; K-26 to L-352; Q-27 to L-352; I-28 to L-352; A-29 to L-352; A-30 to L-352; R-31 to L-352; L-32 to L-352; L-33 to L-352; P-34 to L-352; P-35 to L-352; L-36 to L-352; Y-37 to L-352; S-38 to L-352; L-39 to L-352; V-40 to L-352; F-41 to L-352; I-42 to L-352; F-43 to L-352; G-44 to L-352; F-45 to L-352; V-46 to L-352; G-47 to L-352; N-48 to L-352; M-49 to L-352; L-50 to L-352; V-51 to L-352; I-52 to L-352; L-53 to L-352; I-54 to L-352; L-55 to L-352; I-56 to L-352; N-57 to L-352; C-58 to L-352; K-59 to L-352; R-60 to L-352; L-61 to L-352; K-62 to L-352; S-63 to L-352; M-64 to L-352; T-65 to L-352; D-66 to L-352; I-67 to L-352; Y-68 to L-352; L-69 to L-352; L-70 to L-352; N-71 to L-352; L-72 to L-352; A-73 to L-352; I-74 to L-352; S-75 to L-352; D-76 to L-352; L-77 to L-352; F-78 to L-352; F-79 to L-352; L-80 to L-352; L-81 to L-352; T-82 to L-352; V-83 to L-352; P-84 to L-352; F-85 to L-352; W-86 to L-352; A-87 to L-352; H-88 to L-352; Y-89 to L-352; A-90 to L-352; A-91 to L-352; A-92 to L-352; Q-93 to L-352;

W-94 to L-352; D-95 to L-352; F-96 to L-352; G-97 to L-352; N-98 to L-352; T-99 to L-352; M-100 to L-352; C-101 to L-352; Q-102 to L-352; L-103 to L-352; L-104 to L-352; T-105 to L-352; G-106 to L-352; L-107 to L-352; Y-108 to L-352; F-109 to L-352; I-110 to L-352; G-111 to L-352; F-112 to L-352; F-113 to L-352; S-114 to L-352; G-115 to L-352; I-116 to L-352; F-117 to L-352; F-118 to L-352; I-119 to L-352; I-120 to L-352; L-121 to L-352; L-122 to L-352; T-123 to L-352; I-124 to L-352; D-125 to L-352; R-126 to L-352; Y-127 to L-352; L-128 to L-352; A-129 to L-352; V-130 to L-352; V-131 to L-352; H-132 to L-352; A-133 to L-352; V-134 to L-352; F-135 to L-352; A-136 to L-352; L-137 to L-352; K-138 to L-352; A-139 to L-352; R-140 to L-352; T-141 to L-352; V-142 to L-352; T-143 to L-352; F-144 to L-352; G-145 to L-352; V-146 to L-352; V-147 to L-352; T-148 to L-352; S-149 to L-352; V-150 to L-352; I-151 to L-352; T-152 to L-352; W-153 to L-352; V-154 to L-352; V-155 to L-352; A-156 to L-352; V-157 to L-352; F-158 to L-352; A-159 to L-352; S-160 to L-352; L-161 to L-352; P-162 to L-352; G-163 to L-352; I-164 to L-352; I-165 to L-352; F-166 to L-352; T-167 to L-352; R-168 to L-352; S-169 to L-352; Q-170 to L-352; K-171 to L-352; E-172 to L-352; G-173 to L-352; L-174 to L-352; H-175 to L-352; Y-176 to L-352; T-177 to L-352; C-178 to L-352; S-179 to L-352; S-180 to L-352; H-181 to L-352; F-182 to L-352; P-183 to L-352; Y-184 to L-352; S-185 to L-352; Q-186 to L-352; Y-187 to L-352; Q-188 to L-352; F-189 to L-352; W-190 to L-352; K-191 to L-352; N-192 to L-352; F-193 to L-352; Q-194 to L-352; T-195 to L-352; L-196 to L-352; K-197 to L-352; I-198 to L-352; V-199 to L-352; I-200 to L-352; L-201 to L-352; G-202 to L-352; L-203 to L-352; V-204 to L-352; L-205 to L-352; P-206 to L-352; L-207 to L-352; L-208 to L-352; V-209 to L-352; M-210 to L-352; V-211 to L-352; I-212 to L-352; C-213 to L-352; Y-214 to L-352; S-215 to L-352; G-216 to L-352; I-217 to L-352; L-218 to L-352; K-219 to L-352; T-220 to L-352; L-221 to L-352; L-222 to L-352; R-223 to L-352; C-224 to L-352; R-225 to L-352; N-226 to L-352; E-227 to L-352; K-228 to L-352; K-229 to L-352; R-230 to L-352; H-231 to L-352; R-232 to L-352; A-233 to L-352; V-234 to L-352; R-235 to L-352; L-236 to L-352; I-237 to L-352; F-238 to L-352; T-239 to L-352; I-240 to L-352; M-241 to L-352; I-242 to L-352; V-243 to L-352; Y-244 to L-352; F-245 to L-352; L-246 to L-352; F-247 to L-352; W-248 to L-352; A-249 to L-352; P-250 to L-352; Y-251 to L-352; N-252 to L-352; I-253 to L-352; V-254 to L-352; L-255 to L-352; L-256 to L-352; L-257 to L-352; N-258 to L-352; T-259 to L-352; F-260 to L-352; Q-261 to L-352; E-262 to L-352; F-263 to L-352; F-264 to L-352; G-265 to L-352; L-266 to L-352; N-267 to L-352; N-268 to L-352; C-269 to L-352; S-270

to L-352; S-271 to L-352; S-272 to L-352; N-273 to L-352; R-274 to L-352; L-275 to L-352; D-276 to L-352; Q-277 to L-352; A-278 to L-352; M-279 to L-352; Q-280 to L-352; V-281 to L-352; T-282 to L-352; E-283 to L-352; T-284 to L-352; L-285 to L-352; G-286 to L-352; M-287 to L-352; T-288 to L-352; H-289 to L-352; C-290 to L-352; C-291 to L-352; I-292 to L-352; N-293 to L-352; P-294 to L-352; I-295 to L-352; I-296 to L-352; Y-297 to L-352; A-298 to L-352; F-299 to L-352; V-300 to L-352; G-301 to L-352; E-302 to L-352; K-303 to L-352; F-304 to L-352; R-305 to L-352; N-306 to L-352; Y-307 to L-352; L-308 to L-352; L-309 to L-352; V-310 to L-352; F-311 to L-352; F-312 to L-352; Q-313 to L-352; K-314 to L-352; H-315 to L-352; I-316 to L-352; A-317 to L-352; K-318 to L-352; R-319 to L-352; F-320 to L-352; C-321 to L-352; K-322 to L-352; C-323 to L-352; C-324 to L-352; S-325 to L-352; I-326 to L-352; F-327 to L-352; Q-328 to L-352; Q-329 to L-352; E-330 to L-352; A-331 to L-352; P-332 to L-352; E-333 to L-352; R-334 to L-352; A-335 to L-352; S-336 to L-352; S-337 to L-352; V-338 to L-352; Y-339 to L-352; T-340 to L-352; R-341 to L-352; S-342 to L-352; T-343 to L-352; E-344 to L-352; E-345 to L-352; Q-346 to L-352; and/or E-347 to L-352 of SEQ ID NO:22. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence encoding the G-protein Chemokine Receptor (CCR5) polypeptide described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind G-protein Chemokine Receptor (CCR5) ligand) may still be retained. For example the ability of the shortened G-protein Chemokine Receptor (CCR5) mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that an G-protein Chemokine Receptor (CCR5) mutein with a large number of deleted C-terminal amino acid residues may retain

some biological or immunogenic activities. In fact, peptides composed of as few as six G-protein Chemokine Receptor (CCR5) amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of the G-protein Chemokine Receptor (CCR5) polypeptide shown in FIG. 1 (SEQ ID NO:2) or of the polypeptide encoded by the deposited clone, as described by the general formula 1-n, where n is an integer from 6 to 346, where n corresponds to the position of amino acid residue identified in SEQ ID NO:2 or in the polypeptide encoded by the deposited clone. More in particular, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of D-2 to G-351; D-2 to V-350; D-2 to S-349; D-2 to I-348; D-2 to E-347; D-2 to Q-346; D-2 to E-345; D-2 to G-344; D-2 to T-343; D-2 to S-342; D-2 to R-341; D-2 to T-340; D-2 to Y-339; D-2 to V-338; D-2 to S-337; D-2 to S-336; D-2 to A-335; D-2 to R-334; D-2 to E-333; D-2 to P-332; D-2 to A-331; D-2 to E-330; D-2 to Q-329; D-2 to Q-328; D-2 to F-327; D-2 to I-326; D-2 to S-325; D-2 to C-324; D-2 to C-323; D-2 to K-322; D-2 to C-321; D-2 to F-320; D-2 to R-319; D-2 to K-318; D-2 to A-317; D-2 to I-316; D-2 to H-315; D-2 to K-314; D-2 to Q-313; D-2 to F-312; D-2 to F-311; D-2 to V-310; D-2 to L-309; D-2 to L-308; D-2 to Y-307; D-2 to N-306; D-2 to R-305; D-2 to F-304; D-2 to K-303; D-2 to E-302; D-2 to G-301; D-2 to V-300; D-2 to F-299; D-2 to A-298; D-2 to Y-297; D-2 to I-296; D-2 to I-295; D-2 to P-294; D-2 to N-293; D-2 to I-292; D-2 to C-291; D-2 to C-290; D-2 to H-289; D-2 to T-288; D-2 to M-287; D-2 to G-286; D-2 to L-285; D-2 to T-284; D-2 to E-283; D-2 to T-282; D-2 to V-281; D-2 to Q-280; D-2 to M-279; D-2 to A-278; D-2 to Q-277; D-2 to D-276; D-2 to L-275; D-2 to R-274; D-2 to N-273; D-2 to S-272; D-2 to S-271; D-2 to S-270; D-2 to C-269; D-2 to N-268; D-2 to N-267; D-2 to L-266; D-2 to G-265; D-2 to F-264; D-2 to F-263; D-2 to E-262; D-2 to Q-261; D-2 to F-260; D-2 to T-259; D-2 to N-258; D-2 to L-257; D-2 to L-256; D-2 to L-255; D-2 to V-254; D-2 to I-253; D-2 to N-252; D-2 to Y-251; D-2 to P-250; D-2 to A-249; D-2 to W-248; D-2 to F-247; D-2 to L-246; D-2 to F-245; D-2 to Y-244; D-2 to V-243; D-2 to I-242; D-2 to M-241; D-2 to I-240; D-2 to T-239; D-2 to F-238; D-2 to I-237; D-2 to L-236; D-2 to R-235; D-2 to V-234; D-2 to A-233; D-2 to R-232; D-2 to H-231; D-2 to R-230; D-2 to K-229; D-2 to K-228; D-2 to E-227; D-2 to N-226; D-2 to R-225; D-2 to C-224; D-2 to R-223; D-2 to L-222; D-2 to L-221; D-2 to T-220; D-2 to K-219; D-2 to L-

218; D-2 to I-217; D-2 to G-216; D-2 to S-215; D-2 to Y-214; D-2 to C-213; D-2 to I-212; D-2 to V-211; D-2 to M-210; D-2 to V-209; D-2 to L-208; D-2 to L-207; D-2 to P-206; D-2 to L-205; D-2 to V-204; D-2 to L-203; D-2 to G-202; D-2 to L-201; D-2 to I-200; D-2 to V-199; D-2 to I-198; D-2 to K-197; D-2 to L-196; D-2 to T-195; D-2 to Q-194; D-2 to F-193; D-2 to N-192; D-2 to K-191; D-2 to W-190; D-2 to F-189; D-2 to Q-188; D-2 to Y-187; D-2 to Q-186; D-2 to S-185; D-2 to Y-184; D-2 to P-183; D-2 to F-182; D-2 to H-181; D-2 to S-180; D-2 to S-179; D-2 to C-178; D-2 to T-177; D-2 to Y-176; D-2 to H-175; D-2 to L-174; D-2 to G-173; D-2 to E-172; D-2 to K-171; D-2 to Q-170; D-2 to S-169; D-2 to R-168; D-2 to T-167; D-2 to F-166; D-2 to I-165; D-2 to I-164; D-2 to G-163; D-2 to P-162; D-2 to L-161; D-2 to S-160; D-2 to A-159; D-2 to F-158; D-2 to V-157; D-2 to A-156; D-2 to V-155; D-2 to V-154; D-2 to W-153; D-2 to T-152; D-2 to I-151; D-2 to V-150; D-2 to S-149; D-2 to T-148; D-2 to V-147; D-2 to V-146; D-2 to G-145; D-2 to F-144; D-2 to T-143; D-2 to V-142; D-2 to T-141; D-2 to R-140; D-2 to A-139; D-2 to K-138; D-2 to L-137; D-2 to A-136; D-2 to F-135; D-2 to V-134; D-2 to A-133; D-2 to H-132; D-2 to V-131; D-2 to I-130; D-2 to A-129; D-2 to L-128; D-2 to Y-127; D-2 to R-126; D-2 to D-125; D-2 to I-124; D-2 to T-123; D-2 to L-122; D-2 to L-121; D-2 to I-120; D-2 to I-119; D-2 to F-118; D-2 to F-117; D-2 to I-116; D-2 to G-115; D-2 to S-114; D-2 to F-113; D-2 to F-112; D-2 to G-111; D-2 to I-110; D-2 to F-109; D-2 to Y-108; D-2 to L-107; D-2 to G-106; D-2 to T-105; D-2 to L-104; D-2 to L-103; D-2 to Q-102; D-2 to C-101; D-2 to M-100; D-2 to T-99; D-2 to N-98; D-2 to G-97; D-2 to F-96; D-2 to D-95; D-2 to W-94; D-2 to Q-93; D-2 to A-92; D-2 to A-91; D-2 to A-90; D-2 to Y-89; D-2 to H-88; D-2 to A-87; D-2 to W-86; D-2 to F-85; D-2 to P-84; D-2 to V-83; D-2 to T-82; D-2 to L-81; D-2 to L-80; D-2 to F-79; D-2 to F-78; D-2 to L-77; D-2 to D-76; D-2 to S-75; D-2 to I-74; D-2 to A-73; D-2 to L-72; D-2 to N-71; D-2 to L-70; D-2 to L-69; D-2 to Y-68; D-2 to I-67; D-2 to D-66; D-2 to T-65; D-2 to M-64; D-2 to S-63; D-2 to E-62; D-2 to L-61; D-2 to R-60; D-2 to Q-59; D-2 to C-58; D-2 to N-57; D-2 to I-56; D-2 to L-55; D-2 to I-54; D-2 to L-53; D-2 to I-52; D-2 to V-51; D-2 to L-50; D-2 to M-49; D-2 to N-48; D-2 to G-47; D-2 to V-46; D-2 to F-45; D-2 to G-44; D-2 to F-43; D-2 to I-42; D-2 to F-41; D-2 to V-40; D-2 to L-39; D-2 to S-38; D-2 to Y-37; D-2 to L-36; D-2 to P-35; D-2 to P-34; D-2 to L-33; D-2 to L-32; D-2 to R-31; D-2 to A-30; D-2 to A-29; D-2 to I-28; D-2 to Q-27; D-2 to K-26; D-2 to V-25; D-2 to N-24; D-2 to I-23; D-2 to K-22; D-2 to P-21; D-2 to C-20; D-2 to P-19; D-2 to E-18; D-2 to S-17; D-2 to T-16; D-2 to Y-15; D-2 to Y-14; D-2 to N-13; D-2 to I-12; D-2 to D-11; D-2 to Y-

10; D-2 to I-9; and/or D-2 to P-8 of SEQ ID NO:2. Moreover, a methionine may be added to the N-terminus of each of these C-terminal constructs. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Additionally, the invention provides polynucleotides encoding polypeptides comprising, or alternatively consisting of, the amino acid sequence of residues of D-2 to G-351; D-2 to V-350; D-2 to S-349; D-2 to I-348; D-2 to E-347; D-2 to Q-346; D-2 to E-345; D-2 to E-344; D-2 to T-343; D-2 to S-342; D-2 to R-341; D-2 to T-340; D-2 to Y-339; D-2 to V-338; D-2 to S-337; D-2 to S-336; D-2 to A-335; D-2 to R-334; D-2 to E-333; D-2 to P-332; D-2 to A-331; D-2 to E-330; D-2 to Q-329; D-2 to Q-328; D-2 to F-327; D-2 to I-326; D-2 to S-325; D-2 to C-324; D-2 to C-323; D-2 to K-322; D-2 to C-321; D-2 to F-320; D-2 to R-319; D-2 to K-318; D-2 to A-317; D-2 to I-316; D-2 to H-315; D-2 to K-314; D-2 to Q-313; D-2 to F-312; D-2 to F-311; D-2 to V-310; D-2 to L-309; D-2 to L-308; D-2 to Y-307; D-2 to N-306; D-2 to R-305; D-2 to F-304; D-2 to K-303; D-2 to E-302; D-2 to G-301; D-2 to V-300; D-2 to F-299; D-2 to A-298; D-2 to Y-297; D-2 to I-296; D-2 to I-295; D-2 to P-294; D-2 to N-293; D-2 to I-292; D-2 to C-291; D-2 to C-290; D-2 to H-289; D-2 to T-288; D-2 to M-287; D-2 to G-286; D-2 to L-285; D-2 to T-284; D-2 to E-283; D-2 to T-282; D-2 to V-281; D-2 to Q-280; D-2 to M-279; D-2 to A-278; D-2 to Q-277; D-2 to D-276; D-2 to L-275; D-2 to R-274; D-2 to N-273; D-2 to S-272; D-2 to S-271; D-2 to S-270; D-2 to C-269; D-2 to N-268; D-2 to N-267; D-2 to L-266; D-2 to G-265; D-2 to F-264; D-2 to F-263; D-2 to E-262; D-2 to Q-261; D-2 to F-260; D-2 to T-259; D-2 to N-258; D-2 to L-257; D-2 to L-256; D-2 to L-255; D-2 to V-254; D-2 to I-253; D-2 to N-252; D-2 to Y-251; D-2 to P-250; D-2 to A-249; D-2 to W-248; D-2 to F-247; D-2 to L-246; D-2 to F-245; D-2 to Y-244; D-2 to V-243; D-2 to I-242; D-2 to M-241; D-2 to I-240; D-2 to T-239; D-2 to F-238; D-2 to I-237; D-2 to L-236; D-2 to R-235; D-2 to V-234; D-2 to A-233; D-2 to R-232; D-2 to H-231; D-2 to R-230; D-2 to K-229; D-2 to K-228; D-2 to E-227; D-2 to N-226; D-2 to R-225; D-2 to C-224; D-2 to R-223; D-2 to L-222; D-2 to L-221; D-2 to T-220; D-2 to K-219; D-2 to L-218; D-2 to I-217; D-2 to G-216; D-2 to S-215; D-2 to Y-214; D-2 to C-213; D-2 to I-212; D-2 to V-211; D-2 to M-210; D-2 to V-209; D-2 to L-208; D-2 to L-207; D-2 to P-206; D-2 to L-205; D-2 to V-204; D-2 to L-203; D-2 to G-202; D-2 to L-201; D-2 to I-200; D-2 to V-199; D-2 to I-198; D-2 to K-197; D-2 to L-196; D-2 to T-195; D-2 to Q-194; D-2 to F-193; D-2 to N-192; D-2 to K-191; D-2 to W-190; D-2 to F-189; D-2 to Q-188; D-2 to Y-187; D-2 to Q-186; D-2 to S-185; D-2 to Y-184; D-2 to P-183; D-2 to F-

182; D-2 to H-181; D-2 to S-180; D-2 to S-179; D-2 to C-178; D-2 to T-177; D-2 to Y-176; D-2 to H-175; D-2 to L-174; D-2 to G-173; D-2 to E-172; D-2 to K-171; D-2 to Q-170; D-2 to S-169; D-2 to R-168; D-2 to T-167; D-2 to F-166; D-2 to I-165; D-2 to I-164; D-2 to G-163; D-2 to P-162; D-2 to L-161; D-2 to S-160; D-2 to A-159; D-2 to F-158; D-2 to V-157; D-2 to A-156; D-2 to V-155; D-2 to V-154; D-2 to W-153; D-2 to T-152; D-2 to I-151; D-2 to V-150; D-2 to S-149; D-2 to T-148; D-2 to V-147; D-2 to V-146; D-2 to G-145; D-2 to F-144; D-2 to T-143; D-2 to V-142; D-2 to T-141; D-2 to R-140; D-2 to A-139; D-2 to K-138; D-2 to L-137; D-2 to A-136; D-2 to F-135; D-2 to V-134; D-2 to A-133; D-2 to H-132; D-2 to V-131; D-2 to V-130; D-2 to A-129; D-2 to L-128; D-2 to Y-127; D-2 to R-126; D-2 to D-125; D-2 to I-124; D-2 to T-123; D-2 to L-122; D-2 to L-121; D-2 to I-120; D-2 to I-119; D-2 to F-118; D-2 to F-117; D-2 to I-116; D-2 to G-115; D-2 to S-114; D-2 to F-113; D-2 to F-112; D-2 to G-111; D-2 to I-110; D-2 to F-109; D-2 to Y-108; D-2 to L-107; D-2 to G-106; D-2 to T-105; D-2 to L-104; D-2 to L-103; D-2 to Q-102; D-2 to C-101; D-2 to M-100; D-2 to T-99; D-2 to N-98; D-2 to G-97; D-2 to F-96; D-2 to D-95; D-2 to W-94; D-2 to Q-93; D-2 to A-92; D-2 to A-91; D-2 to A-90; D-2 to Y-89; D-2 to H-88; D-2 to A-87; D-2 to W-86; D-2 to F-85; D-2 to P-84; D-2 to V-83; D-2 to T-82; D-2 to L-81; D-2 to L-80; D-2 to F-79; D-2 to F-78; D-2 to L-77; D-2 to D-76; D-2 to S-75; D-2 to I-74; D-2 to A-73; D-2 to L-72; D-2 to N-71; D-2 to L-70; D-2 to L-69; D-2 to Y-68; D-2 to I-67; D-2 to D-66; D-2 to T-65; D-2 to M-64; D-2 to S-63; D-2 to K-62; D-2 to L-61; D-2 to R-60; D-2 to K-59; D-2 to C-58; D-2 to N-57; D-2 to I-56; D-2 to L-55; D-2 to I-54; D-2 to L-53; D-2 to I-52; D-2 to V-51; D-2 to L-50; D-2 to M-49; D-2 to N-48; D-2 to G-47; D-2 to V-46; D-2 to F-45; D-2 to G-44; D-2 to F-43; D-2 to I-42; D-2 to F-41; D-2 to V-40; D-2 to L-39; D-2 to S-38; D-2 to Y-37; D-2 to L-36; D-2 to P-35; D-2 to P-34; D-2 to L-33; D-2 to L-32; D-2 to R-31; D-2 to A-30; D-2 to A-29; D-2 to I-28; D-2 to Q-27; D-2 to K-26; D-2 to V-25; D-2 to N-24; D-2 to I-23; D-2 to K-22; D-2 to Q-21; D-2 to C-20; D-2 to P-19; D-2 to E-18; D-2 to S-17; D-2 to T-16; D-2 to Y-15; D-2 to Y-14; D-2 to N-13; D-2 to I-12; D-2 to D-11; D-2 to Y-10; D-2 to I-9; and/or D-2 to P-8 of SEQ ID NO:22. Moreover, a methionine may be added to the N-terminus of each of these C-terminal constructs. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence encoding the G-protein

Chemokine Receptor (CCR5) polypeptide described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence.

In addition, any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted G-protein Chemokine Receptor (CCR5) polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO:2 or of the polypeptide encoded by the deposited clone, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also included are a nucleotide sequence encoding a polypeptide consisting of a portion of the complete G-protein Chemokine Receptor (CCR5) amino acid sequence encoded by the clone contained in ATCC Deposit No. 97183, where this portion excludes any integer of amino acid residues from 1 to about 342 amino acids from the amino terminus of the complete amino acid sequence encoded by the clone contained in ATCC Deposit No. 97183, or any integer of amino acid residues from 1 to about 342 amino acids from the carboxy terminus, or any combination of the above amino terminal and carboxy terminal deletions, of the complete amino acid sequence encoded by the clone contained in ATCC Deposit No. 97183. Polynucleotides encoding all of the above deletion mutant polypeptide forms also are provided.

The present application is also directed to proteins containing polypeptides at least 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the G-protein Chemokine Receptor (CCR5) polypeptide sequence set forth herein m-n. In preferred embodiments, the application is directed to proteins containing polypeptides at least 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific G-protein Chemokine Receptor (CCR5) N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Additional preferred polypeptide fragments comprise, or alternatively consist of, the amino acid sequence of residues: M-1 to Y-15; D-2 to T-16; Y-3 to S-17; Q-4 to E-18; V-5 to P-19; S-6 to C-20; S-7 to P-21; P-8 to K-22; I-9 to I-23; Y-10 to N-24; D-11 to V-25; I-12 to K-26; N-13 to Q-27; Y-14 to I-28; Y-15 to A-29; T-16 to A-30; S-17 to R-31; E-18 to L-32; P-19 to L-33; C-20 to P-34; P-21 to P-35; K-22 to L-36; I-23 to Y-37; N-24

to S-38; V-25 to L-39; K-26 to V-40; Q-27 to F-41; I-28 to I-42; A-29 to F-43; A-30 to G-44; R-31 to F-45; L-32 to V-46; L-33 to G-47; P-34 to N-48; P-35 to M-49; L-36 to L-50; Y-37 to V-51; S-38 to I-52; L-39 to L-53; V-40 to I-54; F-41 to L-55; I-42 to I-56; F-43 to N-57; G-44 to C-58; F-45 to Q-59; V-46 to R-60; G-47 to L-61; N-48 to E-62; M-49 to S-63; L-50 to M-64; V-51 to T-65; I-52 to D-66; L-53 to I-67; I-54 to Y-68; L-55 to L-69; I-56 to L-70; N-57 to N-71; C-58 to L-72; Q-59 to A-73; R-60 to I-74; L-61 to S-75; E-62 to D-76; S-63 to L-77; M-64 to F-78; T-65 to F-79; D-66 to L-80; I-67 to L-81; Y-68 to T-82; L-69 to V-83; L-70 to P-84; N-71 to F-85; L-72 to W-86; A-73 to A-87; I-74 to H-88; S-75 to Y-89; D-76 to A-90; L-77 to A-91; F-78 to A-92; F-79 to Q-93; L-80 to W-94; L-81 to D-95; T-82 to F-96; V-83 to G-97; P-84 to N-98; F-85 to T-99; W-86 to M-100; A-87 to C-101; H-88 to Q-102; Y-89 to L-103; A-90 to L-104; A-91 to T-105; A-92 to G-106; Q-93 to L-107; W-94 to Y-108; D-95 to F-109; F-96 to I-110; G-97 to G-111; N-98 to F-112; T-99 to F-113; M-100 to S-114; C-101 to G-115; Q-102 to I-116; L-103 to F-117; L-104 to F-118; T-105 to I-119; G-106 to I-120; L-107 to L-121; Y-108 to L-122; F-109 to T-123; I-110 to I-124; G-111 to D-125; F-112 to R-126; F-113 to Y-127; S-114 to L-128; G-115 to A-129; I-116 to I-130; F-117 to V-131; F-118 to H-132; I-119 to A-133; I-120 to V-134; L-121 to F-135; L-122 to A-136; T-123 to L-137; I-124 to K-138; D-125 to A-139; R-126 to R-140; Y-127 to T-141; L-128 to V-142; A-129 to T-143; I-130 to F-144; V-131 to G-145; H-132 to V-146; A-133 to V-147; V-134 to T-148; F-135 to S-149; A-136 to V-150; L-137 to I-151; K-138 to T-152; A-139 to W-153; R-140 to V-154; T-141 to V-155; V-142 to A-156; T-143 to V-157; F-144 to F-158; G-145 to A-159; V-146 to S-160; V-147 to L-161; T-148 to P-162; S-149 to G-163; V-150 to I-164; I-151 to I-165; T-152 to F-166; W-153 to T-167; V-154 to R-168; V-155 to S-169; A-156 to Q-170; V-157 to K-171; F-158 to E-172; A-159 to G-173; S-160 to L-174; L-161 to H-175; P-162 to Y-176; G-163 to T-177; I-164 to C-178; I-165 to S-179; F-166 to S-180; T-167 to H-181; R-168 to F-182; S-169 to P-183; Q-170 to Y-184; K-171 to S-185; E-172 to Q-186; G-173 to Y-187; L-174 to Q-188; H-175 to F-189; Y-176 to W-190; T-177 to K-191; C-178 to N-192; S-179 to F-193; S-180 to Q-194; H-181 to T-195; F-182 to L-196; P-183 to K-197; Y-184 to I-198; S-185 to V-199; Q-186 to I-200; Y-187 to L-201; Q-188 to G-202; F-189 to L-203; W-190 to V-204; K-191 to L-205; N-192 to P-206; F-193 to L-207; Q-194 to L-208; T-195 to V-209; L-196 to M-210; K-197 to V-211; I-198 to I-212; V-199 to C-213; I-200 to Y-214; L-201 to S-215; G-202 to G-216; L-203 to I-217; V-204 to L-218; L-205 to K-219; P-206 to T-220; L-207 to L-221; L-208 to L-222; V-209 to R-

223; M-210 to C-224; V-211 to R-225; I-212 to N-226; C-213 to E-227; Y-214 to K-228; S-215 to K-229; G-216 to R-230; I-217 to H-231; L-218 to R-232; K-219 to A-233; T-220 to V-234; L-221 to R-235; L-222 to L-236; R-223 to I-237; C-224 to F-238; R-225 to T-239; N-226 to I-240; E-227 to M-241; K-228 to I-242; K-229 to V-243; R-230 to Y-244; H-231 to F-245; R-232 to L-246; A-233 to F-247; V-234 to W-248; R-235 to A-249; L-236 to P-250; I-237 to Y-251; F-238 to N-252; T-239 to I-253; I-240 to V-254; M-241 to L-255; I-242 to L-256; V-243 to L-257; Y-244 to N-258; F-245 to T-259; L-246 to F-260; F-247 to Q-261; W-248 to E-262; A-249 to F-263; P-250 to F-264; Y-251 to G-265; N-252 to L-266; I-253 to N-267; V-254 to N-268; L-255 to C-269; L-256 to S-270; L-257 to S-271; N-258 to S-272; T-259 to N-273; F-260 to R-274; Q-261 to L-275; E-262 to D-276; F-263 to Q-277; F-264 to A-278; G-265 to M-279; L-266 to Q-280; N-267 to V-281; N-268 to T-282; C-269 to E-283; S-270 to T-284; S-271 to L-285; S-272 to G-286; N-273 to M-287; R-274 to T-288; L-275 to H-289; D-276 to C-290; Q-277 to C-291; A-278 to I-292; M-279 to N-293; Q-280 to P-294; V-281 to I-295; T-282 to I-296; E-283 to Y-297; T-284 to A-298; L-285 to F-299; G-286 to V-300; M-287 to G-301; T-288 to E-302; H-289 to K-303; C-290 to F-304; C-291 to R-305; I-292 to N-306; N-293 to Y-307; P-294 to L-308; I-295 to L-309; I-296 to V-310; Y-297 to F-311; A-298 to F-312; F-299 to Q-313; V-300 to K-314; G-301 to H-315; E-302 to I-316; K-303 to A-317; F-304 to K-318; R-305 to R-319; N-306 to F-320; Y-307 to C-321; L-308 to K-322; L-309 to C-323; V-310 to C-324; F-311 to S-325; F-312 to I-326; Q-313 to F-327; K-314 to Q-328; H-315 to Q-329; I-316 to E-330; A-317 to A-331; K-318 to P-332; R-319 to E-333; F-320 to R-334; C-321 to A-335; K-322 to S-336; C-323 to S-337; C-324 to V-338; S-325 to Y-339; I-326 to T-340; F-327 to R-341; Q-328 to S-342; Q-329 to T-343; E-330 to G-344; A-331 to E-345; P-332 to Q-346; E-333 to E-347; R-334 to I-348; A-335 to S-349; S-336 to V-350; S-337 to G-351; and/or V-338 to L-352 of SEQ ID NO:2.

Additional preferred polypeptide fragments comprise, or alternatively consist of, the amino acid sequence of residues: M-1 to Y-15; D-2 to T-16; Y-3 to S-17; Q-4 to E-18; V-5 to P-19; S-6 to C-20; S-7 to Q-21; P-8 to K-22; I-9 to I-23; Y-10 to N-24; D-11 to V-25; I-12 to K-26; N-13 to Q-27; Y-14 to I-28; Y-15 to A-29; T-16 to A-30; S-17 to R-31; E-18 to L-32; P-19 to L-33; C-20 to P-34; Q-21 to P-35; K-22 to L-36; I-23 to Y-37; N-24 to S-38; V-25 to L-39; K-26 to V-40; Q-27 to F-41; I-28 to I-42; A-29 to F-43; A-30 to G-44; R-31 to F-45; L-32 to V-46; L-33 to G-47; P-34 to N-48; P-35 to M-49; L-36 to L-50; Y-37 to V-51; S-38 to I-52; L-39 to L-53; V-40 to I-54; F-41 to L-55; I-42 to I-56; F-

43 to N-57; G-44 to C-58; F-45 to K-59; V-46 to R-60; G-47 to L-61; N-48 to K-62; M-49 to S-63; L-50 to M-64; V-51 to T-65; I-52 to D-66; L-53 to I-67; I-54 to Y-68; L-55 to L-69; I-56 to L-70; N-57 to N-71; C-58 to L-72; K-59 to A-73; R-60 to I-74; L-61 to S-75; K-62 to D-76; S-63 to L-77; M-64 to F-78; T-65 to F-79; D-66 to L-80; I-67 to L-81; Y-68 to T-82; L-69 to V-83; L-70 to P-84; N-71 to F-85; L-72 to W-86; A-73 to A-87; I-74 to H-88; S-75 to Y-89; D-76 to A-90; L-77 to A-91; F-78 to A-92; F-79 to Q-93; L-80 to W-94; L-81 to D-95; T-82 to F-96; V-83 to G-97; P-84 to N-98; F-85 to T-99; W-86 to M-100; A-87 to C-101; H-88 to Q-102; Y-89 to L-103; A-90 to L-104; A-91 to T-105; A-92 to G-106; Q-93 to L-107; W-94 to Y-108; D-95 to F-109; F-96 to I-110; G-97 to G-111; N-98 to F-112; T-99 to F-113; M-100 to S-114; C-101 to G-115; Q-102 to I-116; L-103 to F-117; L-104 to F-118; T-105 to I-119; G-106 to I-120; L-107 to L-121; Y-108 to L-122; F-109 to T-123; I-110 to I-124; G-111 to D-125; F-112 to R-126; F-113 to Y-127; S-114 to L-128; G-115 to A-129; I-116 to V-130; F-117 to V-131; F-118 to H-132; I-119 to A-133; I-120 to V-134; L-121 to F-135; L-122 to A-136; T-123 to L-137; I-124 to K-138; D-125 to A-139; R-126 to R-140; Y-127 to T-141; L-128 to V-142; A-129 to T-143; V-130 to F-144; V-131 to G-145; H-132 to V-146; A-133 to V-147; V-134 to T-148; F-135 to S-149; A-136 to V-150; L-137 to I-151; K-138 to T-152; A-139 to W-153; R-140 to V-154; T-141 to V-155; V-142 to A-156; T-143 to V-157; F-144 to F-158; G-145 to A-159; V-146 to S-160; V-147 to L-161; T-148 to P-162; S-149 to G-163; V-150 to I-164; I-151 to I-165; T-152 to F-166; W-153 to T-167; V-154 to R-168; V-155 to S-169; A-156 to Q-170; V-157 to K-171; F-158 to E-172; A-159 to G-173; S-160 to L-174; L-161 to H-175; P-162 to Y-176; G-163 to T-177; I-164 to C-178; I-165 to S-179; F-166 to S-180; T-167 to H-181; R-168 to F-182; S-169 to P-183; Q-170 to Y-184; K-171 to S-185; E-172 to Q-186; G-173 to Y-187; L-174 to Q-188; H-175 to F-189; Y-176 to W-190; T-177 to K-191; C-178 to N-192; S-179 to F-193; S-180 to Q-194; H-181 to T-195; F-182 to L-196; P-183 to K-197; Y-184 to I-198; S-185 to V-199; Q-186 to I-200; Y-187 to L-201; Q-188 to G-202; F-189 to L-203; W-190 to V-204; K-191 to L-205; N-192 to P-206; F-193 to L-207; Q-194 to L-208; T-195 to V-209; L-196 to M-210; K-197 to V-211; I-198 to I-212; V-199 to C-213; I-200 to Y-214; L-201 to S-215; G-202 to G-216; L-203 to I-217; V-204 to L-218; L-205 to K-219; P-206 to T-220; L-207 to L-221; L-208 to L-222; V-209 to R-223; M-210 to C-224; V-211 to R-225; I-212 to N-226; C-213 to E-227; Y-214 to K-228; S-215 to K-229; G-216 to R-230; I-217 to H-231; L-218 to R-232; K-219 to A-233; T-220 to V-234; L-221 to R-235; L-222 to L-236; R-223 to I-237; C-224 to F-

238; R-225 to T-239; N-226 to I-240; E-227 to M-241; K-228 to I-242; K-229 to V-243; R-230 to Y-244; H-231 to F-245; R-232 to L-246; A-233 to F-247; V-234 to W-248; R-235 to A-249; L-236 to P-250; I-237 to Y-251; F-238 to N-252; T-239 to I-253; I-240 to V-254; M-241 to L-255; I-242 to L-256; V-243 to L-257; Y-244 to N-258; F-245 to T-259; L-246 to F-260; F-247 to Q-261; W-248 to E-262; A-249 to F-263; P-250 to F-264; Y-251 to G-265; N-252 to L-266; I-253 to N-267; V-254 to N-268; L-255 to C-269; L-256 to S-270; L-257 to S-271; N-258 to S-272; T-259 to N-273; F-260 to R-274; Q-261 to L-275; E-262 to D-276; F-263 to Q-277; F-264 to A-278; G-265 to M-279; L-266 to Q-280; N-267 to V-281; N-268 to T-282; C-269 to E-283; S-270 to T-284; S-271 to L-285; S-272 to G-286; N-273 to M-287; R-274 to T-288; L-275 to H-289; D-276 to C-290; Q-277 to C-291; A-278 to I-292; M-279 to N-293; Q-280 to P-294; V-281 to I-295; T-282 to I-296; E-283 to Y-297; T-284 to A-298; L-285 to F-299; G-286 to V-300; M-287 to G-301; T-288 to E-302; H-289 to K-303; C-290 to F-304; C-291 to R-305; I-292 to N-306; N-293 to Y-307; P-294 to L-308; I-295 to L-309; I-296 to V-310; Y-297 to F-311; A-298 to F-312; F-299 to Q-313; V-300 to K-314; G-301 to H-315; E-302 to I-316; K-303 to A-317; F-304 to K-318; R-305 to R-319; N-306 to F-320; Y-307 to C-321; L-308 to K-322; L-309 to C-323; V-310 to C-324; F-311 to S-325; F-312 to I-326; Q-313 to F-327; K-314 to Q-328; H-315 to Q-329; I-316 to E-330; A-317 to A-331; K-318 to P-332; R-319 to E-333; F-320 to R-334; C-321 to A-335; K-322 to S-336; C-323 to S-337; C-324 to V-338; S-325 to Y-339; I-326 to T-340; F-327 to R-341; Q-328 to S-342; Q-329 to T-343; E-330 to E-344; A-331 to E-345; P-332 to Q-346; E-333 to E-347; R-334 to I-348; A-335 to S-349; S-336 to V-350; S-337 to G-351; and/or V-338 to L-352 of SEQ ID NO:22.

These polypeptide fragments may retain the biological activity of G-protein Chemokine Receptor (CCR5) polypeptides of the invention and/or may be useful to generate or screen for antibodies, as described further below. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

The present application is also directed to nucleic acid molecules comprising, or alternatively, consisting of, a polynucleotide sequence at least 90%, 92%, 95%, 96%, 97%, 98%, or 99% identical to the polynucleotide sequence encoding the G-protein Chemokine Receptor (CCR5) polypeptide described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence.

Additionally, the present application is also directed to proteins containing polypeptides at least 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the

G-protein Chemokine Receptor (CCR5) polypeptide fragments set forth above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferably, the polynucleotide fragments of the invention encode a polypeptide which demonstrates a G-protein Chemokine Receptor (CCR5) functional activity. By a polypeptide demonstrating a G-protein Chemokine Receptor (CCR5) "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) G-protein Chemokine Receptor (CCR5) protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a G-protein Chemokine Receptor (CCR5) polypeptide for binding) to an anti-G-protein Chemokine Receptor (CCR5) antibody], immunogenicity (ability to generate antibody which binds to a G-protein Chemokine Receptor (CCR5) polypeptide), ability to form multimers with G-protein Chemokine Receptor (CCR5) polypeptides of the invention, and ability to bind to a receptor or ligand for a G-protein Chemokine Receptor (CCR5) polypeptide.

The functional activity of G-protein Chemokine Receptor (CCR5) polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length G-protein Chemokine Receptor (CCR5) polypeptide for binding to anti-G-protein Chemokine Receptor (CCR5) antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a G-protein Chemokine Receptor (CCR5) ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., 1995, *Microbiol. Rev.* 59:94-123. In another embodiment, physiological correlates of G-protein Chemokine Receptor (CCR5) binding to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of G-protein Chemokine Receptor (CCR5) polypeptides and fragments, variants derivatives and analogs thereof to elicit G-protein Chemokine Receptor (CCR5) related biological activity (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

Among the especially preferred fragments of the invention are fragments characterized by structural or functional attributes of G-protein Chemokine Receptor. Such fragments include amino acid residues that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, surface forming regions, and high antigenic index regions (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) of complete (i.e., full-length) G-protein Chemokine Receptor (CCR5) (SEQ ID NO:2) or encoded by the deposited clone. Certain preferred regions are those set out in FIG. 3 and include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence depicted in FIG. 1 (SEQ ID NO:2) or encoded by the deposited clone, such preferred regions include; Garnier-Robson predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman predicted alpha-regions, beta-regions, turn-regions, and coil-regions; Kyte-Doolittle predicted hydrophilic and hydrophobic regions; Eisenberg alpha and beta amphipathic regions; Emini surface-forming regions; and Jameson-Wolf high antigenic index regions, as predicted using the default parameters of these computer programs. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In additional embodiments, the polynucleotides of the invention encode functional attributes of G-protein Chemokine Receptor. Preferred embodiments of the invention in this regard include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions and high antigenic index regions of G-protein Chemokine Receptor.

The data representing the structural or functional attributes of G-protein Chemokine Receptor (CCR5) set forth in FIG. 1 or encoded by the deposited clone and/or Table 1, as described above, was generated using the various modules and algorithms of the DNA*STAR set on default parameters. In a preferred embodiment, the data presented in columns VIII, IX, XIII, and XIV of Table 1 can be used to determine regions of G-protein Chemokine Receptor (CCR5) which exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from the data presented in columns VIII, IX, XIII, and/or IV by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Certain preferred regions in these regards are set out in FIG. 3, but may, as shown in Table 1, be represented or identified by using tabular representations of the data presented in FIG. 3. The DNA*STAR computer algorithm used to generate FIG. 3 (set on the original default parameters) was used to present the data in FIG. 3 in a tabular format (See Table 1). The tabular format of the data in FIG. 3 may be used to easily determine specific boundaries of a preferred region.

The above-mentioned preferred regions set out in FIG. 3 and in Table 1 include, but are not limited to, regions of the aforementioned types identified by analysis of the amino acid sequence set out in FIG. 1 or encoded by the deposited clone. As set out in FIG. 3 and in Table 1, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and coil-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index.

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Table 1

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Met	1	.	.	B	.	.	.	.	0.24	0.06	.	*	.	0.05	1.68
Asp	2	.	.	B	B	.	.	.	0.33	0.27	.	*	.	-0.30	0.98
Tyr	3	.	.	B	B	.	.	.	0.42	0.23	.	*	.	-0.15	1.02
Gln	4	.	.	B	B	.	.	.	0.60	0.19	.	*	.	-0.15	1.38
Val	5	.	.	B	B	.	.	.	0.10	0.00	.	*	F	0.00	1.28
Ser	6	.	.	B	B	.	.	.	0.46	0.69	*	*	F	-0.45	0.57
Ser	7	.	.	B	B	.	.	.	0.46	0.69	*	*	F	-0.45	0.52
Pro	8	.	.	B	B	.	.	.	-0.19	0.29	*	*	F	0.00	1.17
Ile	9	.	.	B	B	.	.	.	-0.19	0.33	*	*	.	-0.30	0.61
Tyr	10	.	.	B	B	.	.	.	0.42	0.34	*	.	.	-0.30	0.73
Asp	11	.	.	B	.	.	T	.	0.48	0.71	.	.	.	-0.20	0.74
Ile	12	.	.	B	.	.	T	.	0.47	1.04	.	.	.	-0.05	1.66
Asn	13	.	.	B	.	.	T	.	0.38	0.84	*	.	.	-0.05	1.53
Tyr	14	.	.	B	.	.	T	.	1.27	0.47	*	.	.	-0.05	1.23
Tyr	15	.	.	.	B	T	.	.	1.30	0.47	.	*	.	-0.05	3.03
Thr	16	.	.	.	B	T	.	.	0.63	0.21	.	*	F	0.65	2.91
Ser	17	.	.	.	B	T	.	.	1.31	0.39	.	*	F	0.75	1.00
Glu	18	.	.	B	.	.	.	.	1.36	0.06	.	*	F	0.80	0.98
Pro	19	.	.	.	.	T	.	.	0.71	-0.70	.	*	F	2.50	1.36
Cys	20	.	.	.	.	T	T	.	0.96	-0.50	.	*	F	2.50	0.71
Pro	21	.	.	.	.	T	T	.	0.41	-0.49	.	*	F	2.25	0.66
Lys	22	.	.	.	.	T	T	.	0.76	0.16	.	*	F	1.40	0.32
Ile	23	A	.	.	.	.	T	.	0.76	-0.27	*	*	F	1.50	1.19
Asn	24	A	A	.	.	.	.	.	0.08	-0.44	.	*	F	0.85	1.33
Val	25	A	A	.	.	.	.	.	0.16	-0.19	.	*	.	0.30	0.47
Lys	26	.	A	B	.	.	.	.	-0.22	0.31	.	*	.	-0.30	0.67
Gln	27	.	A	B	.	.	.	.	-0.16	0.13	*	*	.	-0.30	0.42
Ile	28	.	A	B	.	.	.	.	-0.08	-0.27	*	*	.	0.45	1.11
Ala	29	.	A	B	.	.	.	.	-0.89	-0.23	*	*	.	0.30	0.46
Ala	30	.	A	B	.	.	.	.	-0.24	0.46	*	*	.	-0.60	0.22
Arg	31	.	A	B	.	.	.	.	-0.50	0.49	*	*	.	-0.60	0.48
Leu	32	.	A	B	.	.	.	.	-1.31	0.23	*	*	.	-0.30	0.74
Leu	33	.	A	B	.	.	.	.	-0.67	0.41	*	*	.	-0.60	0.60
Pro	34	.	.	.	.	.	T	C	-0.38	0.67	*	*	F	0.15	0.48
Pro	35	.	.	.	.	T	T	.	-0.60	1.06	*	*	F	0.35	0.78
Leu	36	.	.	B	.	.	T	.	-1.57	1.06	*	*	.	-0.20	0.78
Tyr	37	.	.	B	.	.	T	.	-1.46	1.01	.	.	.	-0.20	0.38
Ser	38	.	.	B	B	.	.	.	-1.53	1.37	.	.	.	-0.60	0.21
Leu	39	.	.	B	B	.	.	.	-2.02	1.63	.	.	.	-0.60	0.18
Val	40	.	.	B	B	.	.	.	-2.16	1.73	.	.	.	-0.60	0.10
Phe	41	.	.	B	B	.	.	.	-2.04	1.40	.	.	.	-0.60	0.07
Ile	42	.	.	B	B	.	.	.	-2.66	1.80	.	.	.	-0.60	0.08
Phe	43	.	.	B	B	.	.	.	-2.70	1.76	.	.	.	-0.60	0.08
Gly	44	.	.	B	B	.	.	.	-1.89	1.54	.	.	.	-0.60	0.09
Phe	45	.	.	.	B	T	.	.	-1.63	1.16	.	.	.	-0.20	0.20
Val	46	.	.	.	B	.	.	C	-1.74	1.09	.	.	.	-0.40	0.23
Gly	47	.	.	.	B	.	.	C	-1.71	0.99	.	.	.	-0.40	0.19
Asn	48	A	.	.	B	.	.	.	-1.90	1.20	.	.	.	-0.60	0.16
Met	49	A	.	.	B	.	.	.	-2.37	1.10	.	.	.	-0.60	0.15
Leu	50	A	.	.	B	.	.	.	-2.56	1.14	.	.	.	-0.60	0.13
Val	51	.	.	B	B	.	.	.	-2.51	1.40	.	.	.	-0.60	0.06
Ile	52	.	.	B	B	.	.	.	-3.06	1.69	.	.	.	-0.60	0.05
Leu	53	.	.	B	B	.	.	.	-3.06	1.76	.	.	.	-0.60	0.04
Ile	54	.	.	B	B	.	.	.	-3.12	1.47	.	*	.	-0.60	0.09
Leu	55	.	.	B	B	.	.	.	-2.31	1.40	*	.	.	-0.60	0.07
Ile	56	.	.	B	B	.	.	.	-1.34	1.11	*	.	.	-0.60	0.14

Table 1 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Asn	57	.	.	B	B	.	.	.	-1.27	0.43	.	*	.	-0.60	0.39
Cys	58	.	A	B	B	.	.	.	-0.46	0.43	.	.	.	-0.60	0.39
Gln	59	A	A	.	B	.	.	.	0.13	-0.26	*	.	.	0.30	0.96
Arg	60	A	A	.	B	.	.	.	0.34	-0.56	.	.	F	0.75	0.80
Leu	61	A	A	.	B	.	.	.	0.92	-0.34	.	.	F	0.60	1.47
Glu	62	.	A	B	B	.	.	.	0.92	-0.43	.	*	F	0.60	1.23
Ser	63	.	A	B	.	.	.	.	0.70	-0.83	*	*	F	0.90	1.05
Met	64	.	A	B	B	.	.	.	0.46	-0.14	*	*	F	0.45	0.89
Thr	65	.	A	B	B	.	.	.	-0.47	-0.07	.	*	F	0.45	0.80
Asp	66	A	A	.	B	.	.	.	-0.47	0.61	.	*	.	-0.60	0.50
Ile	67	A	A	.	B	.	.	.	-0.47	0.91	.	.	.	-0.60	0.41
Tyr	68	A	A	.	B	.	.	.	-0.98	0.70	.	.	.	-0.60	0.46
Leu	69	A	A	.	B	.	.	.	-0.97	0.90	.	.	.	-0.60	0.23
Leu	70	A	A	.	B	.	.	.	-1.54	1.40	.	*	.	-0.60	0.33
Asn	71	A	A	.	B	.	.	.	-1.84	1.40	.	.	.	-0.60	0.15
Leu	72	.	A	B	B	.	.	.	-0.96	1.03	*	.	.	-0.60	0.24
Ala	73	A	A	.	B	.	.	.	-1.52	0.34	*	.	.	-0.30	0.48
Ile	74	A	A	.	B	.	.	.	-1.41	0.34	.	.	.	-0.30	0.25
Ser	75	A	A	.	B	.	.	.	-1.30	0.73	.	*	.	-0.60	0.26
Asp	76	A	A	.	B	.	.	.	-2.11	0.83	.	.	.	-0.60	0.22
Leu	77	.	A	B	B	.	.	.	-2.11	1.01	.	.	.	-0.60	0.26
Phe	78	.	A	B	B	.	.	.	-1.83	1.01	.	.	.	-0.60	0.16
Phe	79	.	A	B	B	.	.	.	-1.80	1.11	.	.	.	-0.60	0.14
Leu	80	.	A	B	B	.	.	.	-1.71	1.76	.	*	.	-0.60	0.13
Leu	81	.	A	B	B	.	.	.	-2.41	1.50	.	*	.	-0.60	0.22
Thr	82	.	A	B	B	.	.	.	-1.89	1.50	.	*	.	-0.60	0.22
Val	83	.	A	.	B	.	.	C	-1.78	1.63	.	*	.	-0.40	0.29
Pro	84	A	A	.	B	.	.	.	-1.11	1.44	.	*	.	-0.60	0.35
Phe	85	A	A	.	B	.	.	.	-0.54	1.26	.	.	.	-0.60	0.33
Trp	86	A	A	.	B	.	.	.	-0.32	1.53	.	*	.	-0.60	0.70
Ala	87	A	A	.	B	.	.	.	-0.60	1.39	.	.	.	-0.60	0.45
His	88	A	A	.	B	.	.	.	-0.33	1.46	.	.	.	-0.60	0.53
Tyr	89	A	A	.	B	.	.	.	-0.12	1.17	.	.	.	-0.60	0.51
Ala	90	A	A	.	B	.	.	.	0.29	0.66	.	*	.	-0.60	0.87
Ala	91	A	A	.	.	.	.	.	0.58	1.07	.	*	.	-0.60	0.67
Ala	92	A	A	.	.	.	.	.	0.47	0.57	.	*	.	-0.60	0.72
Gln	93	A	A	.	.	.	.	.	0.16	0.60	.	*	.	-0.60	0.62
Trp	94	A	A	.	.	.	.	.	0.40	0.53	.	*	.	-0.60	0.60
Asp	95	.	.	.	.	T	T	.	0.68	0.43	.	*	.	0.20	0.96
Phe	96	.	.	.	.	T	T	.	0.67	0.41	.	*	.	0.20	0.80
Gly	97	.	.	.	.	T	T	.	0.59	0.63	*	*	F	0.35	0.75
Asn	98	.	.	.	.	T	T	.	0.59	0.29	*	*	F	0.65	0.24
Thr	99	.	.	.	B	T	.	.	0.07	0.69	*	.	.	-0.20	0.48
Met	100	.	.	.	B	T	.	.	-0.74	0.59	*	.	.	-0.20	0.40
Cys	101	.	.	B	B	.	.	.	-0.36	0.84	*	.	.	-0.60	0.21
Gln	102	.	.	B	B	.	.	.	-0.36	0.93	*	.	.	-0.60	0.21
Leu	103	.	.	B	B	.	.	.	-1.17	0.87	*	.	.	-0.60	0.21
Leu	104	.	.	B	B	.	.	.	-1.10	0.94	.	.	.	-0.60	0.32
Thr	105	.	.	B	B	.	.	.	-1.20	1.13	*	.	.	-0.60	0.29
Gly	106	.	.	B	B	.	.	.	-1.42	1.51	*	.	.	-0.60	0.30
Leu	107	.	.	B	B	.	.	.	-1.77	1.51	.	.	.	-0.60	0.26
Tyr	108	.	.	B	B	.	.	.	-1.66	1.26	.	.	.	-0.60	0.18
Phe	109	.	.	B	B	.	.	.	-1.54	1.56	.	.	.	-0.60	0.15
Ile	110	.	.	B	B	.	.	.	-1.53	1.91	.	.	.	-0.60	0.16
Gly	111	.	.	B	B	.	.	.	-1.53	1.61	.	.	.	-0.60	0.14
Phe	112	.	.	B	B	.	.	.	-1.61	1.29	.	.	.	-0.60	0.16

Table 1 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Phe	113	.	.	B	B	.	.	.	-2.07	1.19	.	.	.	-0.60	0.16
Ser	114	.	.	.	B	.	.	C	-2.07	1.29	.	.	.	-0.40	0.14
Gly	115	.	.	.	B	.	.	C	-2.07	1.64	.	.	.	-0.40	0.14
Ile	116	.	.	.	B	.	.	C	-2.61	1.54	.	.	.	-0.40	0.11
Phe	117	.	.	B	B	.	.	.	-2.72	1.44	.	.	.	-0.60	0.06
Phe	118	.	.	B	B	.	.	.	-2.83	1.74	.	.	.	-0.60	0.05
Ile	119	.	.	B	B	.	.	.	-2.84	2.00	.	.	.	-0.60	0.06
Ile	120	.	.	B	B	.	.	.	-3.39	1.80	.	*	.	-0.60	0.10
Leu	121	.	.	B	B	.	.	.	-2.50	1.70	*	.	.	-0.60	0.08
Leu	122	.	.	B	B	.	.	.	-1.69	0.91	*	.	.	-0.60	0.18
Thr	123	A	.	.	B	.	.	.	-1.23	0.23	*	.	.	-0.30	0.52
Ile	124	A	.	.	B	.	.	.	-1.16	0.30	*	.	.	-0.30	0.98
Asp	125	A	.	.	.	.	T	.	-0.86	0.30	*	.	F	0.25	0.98
Arg	126	A	.	.	.	.	T	.	-0.93	0.11	*	.	.	0.10	0.69
Tyr	127	A	.	.	.	.	T	.	-0.98	0.31	*	.	.	0.10	0.69
Leu	128	A	.	.	.	.	T	.	-0.70	0.27	*	.	.	0.10	0.30
Ala	129	A	.	.	B	.	.	.	-0.40	0.77	*	.	.	-0.60	0.21
Ile	130	A	.	.	B	.	.	.	-1.26	1.27	*	.	.	-0.60	0.14
Val	131	A	.	.	B	.	.	.	-2.07	1.16	*	.	.	-0.60	0.12
His	132	A	.	.	B	.	.	.	-2.41	1.26	.	.	.	-0.60	0.11
Ala	133	A	.	.	B	.	.	.	-2.41	1.26	.	*	.	-0.60	0.15
Val	134	A	.	.	B	.	.	.	-1.78	1.26	.	*	.	-0.60	0.17
Phe	135	A	.	.	B	.	.	.	-1.48	0.61	.	*	.	-0.60	0.25
Ala	136	A	.	.	B	.	.	.	-0.51	0.61	.	*	.	-0.60	0.25
Leu	137	A	.	.	B	.	.	.	-0.79	0.11	.	*	.	-0.30	0.65
Lys	138	A	.	.	B	.	.	.	-1.06	-0.04	.	*	F	0.60	1.09
Ala	139	A	.	.	B	.	.	.	-0.51	-0.19	.	*	F	0.45	0.80
Arg	140	A	.	.	B	.	.	.	-0.51	-0.20	.	*	F	0.60	1.40
Thr	141	.	.	B	B	.	.	.	-0.27	-0.10	.	*	F	0.45	0.61
Val	142	.	.	B	B	.	.	.	-0.31	0.33	.	*	.	-0.30	0.59
Thr	143	.	.	B	B	.	.	.	-1.21	0.47	.	*	.	-0.60	0.23
Phe	144	.	.	B	B	.	.	.	-0.93	1.11	.	.	.	-0.60	0.12
Gly	145	.	.	B	B	.	.	.	-1.34	1.11	.	.	.	-0.60	0.23
Val	146	.	.	B	B	.	.	.	-1.89	0.86	.	.	.	-0.60	0.21
Val	147	.	.	B	B	.	.	.	-1.92	1.01	.	.	.	-0.60	0.18
Thr	148	.	.	B	B	.	.	.	-1.92	0.91	.	.	.	-0.60	0.13
Ser	149	.	.	B	B	.	.	.	-1.51	0.97	*	.	.	-0.60	0.25
Val	150	.	.	B	B	.	.	.	-2.02	1.24	*	.	.	-0.60	0.35
Ile	151	.	.	B	B	.	.	.	-2.02	1.24	*	.	.	-0.60	0.18
Thr	152	.	.	B	B	.	.	.	-1.76	1.40	*	.	.	-0.60	0.10
Trp	153	.	.	B	B	.	.	.	-2.30	1.51	*	.	.	-0.60	0.14
Val	154	.	.	B	B	.	.	.	-2.70	1.51	*	.	.	-0.60	0.14
Val	155	.	.	B	B	.	.	.	-2.43	1.61	*	.	.	-0.60	0.09
Ala	156	.	.	B	B	.	.	.	-1.84	1.63	*	.	.	-0.60	0.08
Val	157	.	.	B	B	.	.	.	-2.34	1.10	.	.	.	-0.60	0.15
Phe	158	.	.	B	B	.	.	.	-2.27	1.14	.	.	.	-0.60	0.17
Ala	159	.	.	B	B	.	.	.	-1.76	0.93	.	.	.	-0.60	0.25
Ser	160	.	.	.	B	.	.	C	-1.79	0.86	.	.	.	-0.40	0.34
Leu	161	.	.	.	B	.	.	C	-2.09	0.90	.	.	.	-0.40	0.27
Pro	162	.	.	.	B	.	.	C	-1.93	0.80	.	.	.	-0.40	0.19
Gly	163	.	.	.	B	T	.	.	-1.54	1.09	*	.	.	-0.20	0.12
Ile	164	.	.	B	B	.	.	.	-0.84	1.19	.	.	.	-0.60	0.22
Ile	165	.	.	B	B	.	.	.	-0.84	0.50	.	*	.	-0.60	0.27
Phe	166	.	.	B	B	.	.	.	-0.03	0.46	.	.	.	-0.26	0.37
Thr	167	.	.	B	.	.	T	.	0.22	0.43	*	.	F	0.63	0.91
Arg	168	.	.	B	.	.	T	.	0.57	-0.26	*	.	F	2.02	2.60

Table 1 (continued)

Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Ser 169	.	.	.	.	.	T	C	1.11	-0.94	*	.	F	2.86	5.21
Gln 170	.	.	.	.	T	T	.	1.19	-1.30	*	.	F	3.40	3.57
Lys 171	.	.	.	.	T	.	.	1.86	-1.10	*	.	F	2.86	1.50
Glu 172	.	.	.	.	T	.	.	1.92	-0.60	*	.	F	2.52	1.53
Gly 173	.	.	.	B	T	.	.	1.50	-0.23	*	*	F	1.68	1.38
Leu 174	.	.	B	B	.	.	.	1.13	-0.14	.	.	.	0.64	1.00
His 175	.	.	B	B	.	.	.	0.83	0.43	.	.	.	-0.60	0.31
Tyr 176	.	.	B	B	.	.	.	0.49	0.81	.	.	.	-0.60	0.42
Thr 177	.	.	B	B	.	.	.	0.46	0.77	.	*	.	-0.60	0.68
Cys 178	.	.	B	.	.	T	.	0.10	0.59	.	*	.	-0.20	0.68
Ser 179	.	.	.	.	T	T	.	0.70	0.87	.	*	.	0.20	0.38
Ser 180	.	.	.	.	T	T	.	0.49	0.54	.	.	.	0.20	0.40
His 181	.	.	.	.	T	T	.	0.43	0.81	.	.	.	0.35	1.18
Phe 182	.	.	.	.	.	T	C	0.74	0.63	.	.	.	0.15	1.18
Pro 183	.	.	.	.	T	T	.	1.17	0.64	.	.	.	0.35	1.52
Tyr 184	.	.	.	.	T	T	.	1.47	1.01	.	*	.	0.35	1.75
Ser 185	.	.	.	.	T	T	.	1.07	0.91	.	.	.	0.35	3.50
Gln 186	.	.	B	B	.	.	.	0.81	0.91	*	.	.	-0.45	1.96
Tyr 187	.	.	.	B	T	.	.	1.56	1.40	*	.	.	-0.05	1.31
Gln 188	.	.	.	B	T	.	.	1.77	0.64	*	.	.	-0.05	1.96
Phe 189	.	.	.	B	T	.	.	1.31	0.66	*	*	.	-0.05	1.82
Trp 190	.	.	.	B	T	.	.	1.61	1.04	*	*	.	-0.05	1.01
Lys 191	.	.	B	B	.	.	.	1.30	0.69	*	*	.	-0.45	1.01
Asn 192	.	.	.	B	T	.	.	0.73	0.77	*	.	.	-0.05	1.68
Phe 193	.	.	.	B	T	.	.	0.78	0.67	*	*	.	-0.05	1.32
Gln 194	A	.	.	B	.	.	.	0.59	-0.24	*	.	F	0.60	1.32
Thr 195	.	.	.	B	.	.	C	0.02	0.44	*	*	F	-0.25	0.57
Leu 196	.	.	B	B	.	.	.	-0.91	0.69	*	.	.	-0.60	0.49
Lys 197	.	.	B	B	.	.	.	-1.72	0.59	.	.	.	-0.60	0.20
Ile 198	.	.	B	B	.	.	.	-1.37	0.87	*	.	.	-0.60	0.11
Val 199	.	.	B	B	.	.	.	-2.18	0.81	*	*	.	-0.60	0.14
Ile 200	.	.	B	B	.	.	.	-2.72	0.81	.	*	.	-0.60	0.06
Leu 201	.	.	B	B	.	.	.	-2.72	1.46	.	*	.	-0.60	0.06
Gly 202	.	.	B	B	.	.	.	-2.98	1.46	.	*	.	-0.60	0.07
Leu 203	.	.	B	B	.	.	.	-2.90	1.24	.	.	.	-0.60	0.15
Val 204	.	.	B	B	.	.	.	-2.86	1.24	.	.	.	-0.60	0.15
Leu 205	.	.	B	B	.	.	.	-2.82	1.24	.	.	.	-0.60	0.12
Pro 206	.	.	B	B	.	.	.	-2.61	1.46	.	.	.	-0.60	0.11
Leu 207	.	.	B	B	.	.	.	-3.12	1.39	.	.	.	-0.60	0.15
Leu 208	.	.	B	B	.	.	.	-3.20	1.39	.	.	.	-0.60	0.13
Val 209	.	.	B	B	.	.	.	-3.01	1.39	.	.	.	-0.60	0.06
Met 210	.	.	B	B	.	.	.	-2.44	1.53	.	.	.	-0.60	0.04
Val 211	.	.	B	B	.	.	.	-2.53	1.60	.	.	.	-0.60	0.07
Ile 212	.	.	B	B	.	.	.	-2.07	1.30	.	.	.	-0.60	0.13
Cys 213	.	.	B	.	.	T	.	-2.14	1.09	.	.	.	-0.20	0.13
Tyr 214	.	.	B	.	.	T	.	-2.10	1.16	.	.	.	-0.20	0.13
Ser 215	.	.	B	.	.	T	.	-1.46	1.20	.	.	.	-0.20	0.15
Gly 216	.	.	B	.	.	T	.	-0.91	0.51	*	.	.	-0.20	0.55
Ile 217	.	.	B	B	.	.	.	-0.83	0.43	.	.	.	-0.60	0.51
Leu 218	.	.	B	B	.	.	.	-0.98	0.36	*	*	.	-0.30	0.31
Lys 219	.	.	B	B	.	.	.	-0.62	0.66	*	*	F	-0.45	0.26
Thr 220	.	.	B	B	.	.	.	-0.99	0.23	*	*	.	-0.30	0.73
Leu 221	.	.	B	B	.	.	.	-0.53	0.11	*	*	.	-0.30	0.47
Leu 222	A	.	.	B	.	.	.	0.36	-0.57	*	*	.	0.60	0.46
Arg 223	A	.	.	B	.	.	.	1.17	-0.17	*	*	.	0.30	0.52
Cys 224	A	.	.	.	.	T	.	1.17	-0.66	.	*	.	1.15	1.08

Table 1 (continued)

Res Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Arg 225	A	.	.	.	.	T	.	1.52	-1.34	*	*	F	1.30	2.63
Asn 226	A	.	.	.	.	T	.	2.44	-2.03	*	*	F	1.30	2.68
Glu 227	A	.	.	.	.	T	.	3.22	-2.03	.	*	F	1.30	9.80
Lys 228	A	.	.	.	.	.	.	3.22	-2.10	.	*	F	1.10	6.81
Lys 229	A	.	.	.	.	.	.	3.30	-2.10	*	*	F	1.10	8.29
Arg 230	A	.	.	.	.	.	.	2.33	-2.00	*	.	F	1.10	4.83
His 231	A	.	.	B	.	.	.	2.44	-1.36	*	*	.	0.75	1.79
Arg 232	A	.	.	B	.	.	.	1.63	-1.36	*	.	.	0.75	1.76
Ala 233	A	.	.	B	.	.	.	0.70	-0.67	*	*	.	0.60	0.74
Val 234	A	.	.	B	.	.	.	-0.04	0.01	*	.	.	-0.30	0.38
Arg 235	A	.	.	B	.	.	.	-0.47	0.30	*	*	.	-0.30	0.17
Leu 236	.	.	B	B	.	.	.	-1.32	0.79	*	*	.	-0.60	0.24
Ile 237	.	.	B	B	.	.	.	-2.03	0.97	*	*	.	-0.60	0.23
Phe 238	.	.	B	B	.	.	.	-2.33	0.94	*	*	.	-0.60	0.11
Thr 239	.	.	B	B	.	.	.	-2.33	1.63	*	*	.	-0.60	0.10
Ile 240	.	.	B	B	.	.	.	-2.69	1.59	*	*	.	-0.60	0.10
Met 241	.	.	B	B	.	.	.	-2.58	1.66	.	.	.	-0.60	0.19
Ile 242	.	.	B	B	.	.	.	-2.50	1.66	.	.	.	-0.60	0.11
Val 243	.	.	B	B	.	.	.	-2.50	1.86	.	.	.	-0.60	0.13
Tyr 244	.	.	B	B	.	.	.	-2.48	1.96	.	.	.	-0.60	0.12
Phe 245	.	.	B	B	.	.	.	-2.18	2.26	.	.	.	-0.60	0.17
Leu 246	.	.	B	B	.	.	.	-1.79	2.07	.	.	.	-0.60	0.24
Phe 247	.	.	.	B	T	.	.	-1.14	1.86	.	.	.	-0.20	0.23
Trp 248	.	.	.	B	.	.	C	-0.29	1.86	.	*	.	-0.40	0.42
Ala 249	.	.	.	.	.	T	C	-0.93	1.47	.	.	.	0.00	0.82
Pro 250	.	.	.	.	.	T	C	-1.09	1.47	.	*	.	0.00	0.67
Tyr 251	.	.	.	.	T	T	.	-1.09	1.33	.	.	.	0.20	0.47
Asn 252	.	.	B	.	.	T	.	-1.20	1.10	.	*	.	-0.20	0.38
Ile 253	.	.	B	B	.	.	.	-1.72	1.29	.	*	.	-0.60	0.20
Val 254	.	.	B	B	.	.	.	-1.13	1.54	.	*	.	-0.60	0.11
Leu 255	.	.	B	B	.	.	.	-1.23	1.19	*	.	.	-0.60	0.11
Leu 256	.	.	B	B	.	.	.	-1.69	1.27	*	.	.	-0.60	0.22
Leu 257	.	.	B	B	.	.	.	-1.69	1.37	*	.	.	-0.60	0.26
Asn 258	.	.	B	B	.	.	.	-0.80	1.13	*	.	.	-0.60	0.54
Thr 259	A	.	.	B	.	.	.	-0.64	0.44	*	.	.	-0.45	1.14
Phe 260	A	.	.	B	.	.	.	-0.53	0.54	*	.	.	-0.45	1.20
Gln 261	.	.	B	B	.	.	.	-0.07	0.64	*	.	.	-0.60	0.64
Glu 262	.	.	B	B	.	.	.	-0.07	0.67	*	.	.	-0.60	0.44
Phe 263	.	.	B	.	.	.	.	-0.07	0.87	*	.	.	-0.40	0.42
Phe 264	.	.	.	.	T	.	.	0.24	0.49	.	.	.	0.00	0.39
Gly 265	.	.	.	.	T	.	.	0.28	0.49	.	.	.	0.00	0.36
Leu 266	.	.	.	.	T	.	.	-0.02	1.06	.	.	.	0.00	0.22
Asn 267	.	.	.	.	T	.	.	-0.32	0.66	.	.	.	0.00	0.35
Asn 268	.	.	.	.	T	.	.	0.08	0.26	.	.	F	0.45	0.47
Cys 269	.	.	.	.	T	T	.	0.78	0.21	*	*	F	0.65	0.76
Ser 270	.	.	.	.	T	T	.	1.23	-0.07	*	*	F	1.25	0.76
Ser 271	.	.	.	.	T	T	.	1.23	-0.47	.	*	F	1.25	0.93
Ser 272	.	.	.	.	.	T	C	1.23	-0.19	*	*	F	1.20	1.43
Asn 273	.	A	.	.	T	.	.	1.23	-0.76	.	*	F	1.30	1.79
Arg 274	.	A	.	.	T	.	.	1.31	-0.74	*	*	F	1.30	2.31
Leu 275	A	A	.	.	.	.	.	1.01	-0.63	*	*	F	0.90	1.74
Asp 276	A	A	.	.	.	.	.	1.31	-0.40	*	*	F	0.60	1.07
Gln 277	A	A	.	.	.	.	.	0.76	-0.40	*	*	.	0.30	0.95
Ala 278	A	.	.	B	.	.	.	0.44	0.24	*	*	.	-0.30	0.85
Met 279	.	.	B	B	.	.	.	0.33	0.04	*	*	.	-0.30	0.74
Gln 280	.	.	B	B	.	.	.	0.83	0.04	*	*	.	-0.30	0.74

Table 1 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Val	281	.	.	B	B	.	.	.	0.02	0.13	*	.	.	-0.15	1.05
Thr	282	A	.	.	B	.	.	.	-0.32	0.31	*	.	F	-0.15	0.88
Glu	283	A	.	.	B	.	.	.	-0.33	0.13	*	.	F	-0.15	0.50
Thr	284	A	.	.	B	.	.	.	-0.04	0.34	.	*	F	-0.15	0.67
Leu	285	A	.	.	B	.	.	.	-0.08	0.19	.	.	.	-0.30	0.67
Gly	286	.	.	.	B	T	.	.	0.11	0.20	.	.	.	0.10	0.52
Met	287	.	.	.	B	T	.	.	-0.24	0.77	.	.	.	-0.20	0.19
Thr	288	.	.	B	B	.	.	.	-1.13	0.86	.	.	.	-0.60	0.13
His	289	.	.	B	B	.	.	.	-0.82	0.86	.	.	.	-0.60	0.09
Cys	290	.	.	B	B	.	.	.	-0.22	0.83	.	.	.	-0.60	0.15
Cys	291	.	.	B	B	.	.	.	-0.77	0.64	.	.	.	-0.60	0.16
Ile	292	.	.	B	B	.	.	.	-1.06	0.84	.	.	.	-0.60	0.08
Asn	293	.	.	B	B	.	.	.	-0.99	1.03	*	*	.	-0.60	0.11
Pro	294	.	.	B	B	.	.	.	-1.54	1.21	*	.	.	-0.60	0.31
Ile	295	.	A	B	B	.	.	.	-1.58	1.14	*	.	.	-0.60	0.44
Ile	296	.	A	B	B	.	.	.	-1.77	1.24	*	.	.	-0.60	0.24
Tyr	297	.	A	B	B	.	.	.	-1.22	1.49	*	.	.	-0.60	0.11
Ala	298	.	A	B	B	.	.	.	-1.22	1.49	.	.	.	-0.60	0.16
Phe	299	.	A	B	B	.	.	.	-0.97	0.80	.	.	.	-0.60	0.40
Val	300	.	A	B	B	.	.	.	-0.78	0.11	*	*	.	-0.30	0.51
Gly	301	A	A	.	B	.	.	.	0.22	0.14	*	*	F	-0.15	0.44
Glu	302	A	A	.	.	.	.	.	0.47	-0.36	*	*	F	0.45	0.99
Lys	303	A	A	.	.	.	.	.	0.81	-0.74	*	*	F	0.90	2.14
Phe	304	A	.	.	.	.	T	.	0.70	-0.63	*	*	F	1.30	3.39
Arg	305	A	.	.	.	.	T	.	0.74	-0.37	*	*	.	0.85	1.62
Asn	306	A	.	.	.	.	T	.	0.23	0.31	*	*	.	0.10	0.67
Tyr	307	A	.	.	.	.	T	.	-0.47	0.96	*	*	.	-0.20	0.57
Leu	308	A	.	.	B	.	.	.	-1.21	0.96	*	*	.	-0.60	0.25
Leu	309	A	.	.	B	.	.	.	-0.51	1.74	*	*	.	-0.60	0.14
Val	310	A	.	.	B	.	.	.	-0.58	1.74	.	.	.	-0.60	0.15
Phe	311	A	.	.	B	.	.	.	-0.61	0.99	*	.	.	-0.60	0.36
Phe	312	A	.	.	B	.	.	.	-1.26	0.80	*	.	.	-0.60	0.60
Gln	313	A	.	.	B	.	.	.	-1.03	0.80	*	.	.	-0.60	0.57
Lys	314	A	.	.	B	.	.	.	-0.18	0.66	*	.	.	-0.60	0.66
His	315	A	A	.	.	.	.	.	0.79	-0.13	*	*	.	0.45	1.53
Ile	316	A	A	.	.	.	.	.	0.79	-0.91	*	.	.	0.75	1.73
Ala	317	A	A	.	.	.	.	.	0.82	-0.53	*	.	.	0.88	0.75
Lys	318	A	A	.	.	.	.	.	0.87	0.04	*	.	.	0.26	0.30
Arg	319	.	A	.	.	T	.	.	0.16	-0.46	*	.	.	1.54	0.84
Phe	320	.	A	.	.	T	.	.	-0.48	-0.57	*	.	.	2.12	0.45
Cys	321	.	.	.	.	T	T	.	0.11	-0.50	*	.	.	2.80	0.12
Lys	322	.	.	.	.	T	T	.	-0.19	-0.11	*	.	.	2.22	0.08
Cys	323	.	.	.	.	T	T	.	-0.93	0.57	*	.	.	1.04	0.07
Cys	324	.	.	.	.	T	T	.	-1.04	0.57	*	.	.	0.76	0.11
Ser	325	.	A	.	.	T	.	.	-0.34	0.40	*	.	.	0.38	0.09
Ile	326	.	A	B	.	.	.	.	0.32	0.80	*	.	.	-0.60	0.30
Phe	327	.	A	B	.	.	.	.	-0.31	0.23	*	.	.	-0.30	0.97
Gln	328	A	A	.	.	.	.	.	0.14	0.16	.	.	F	-0.15	0.73
Gln	329	A	A	.	.	.	.	.	0.81	0.20	.	*	F	0.00	1.61
Glu	330	A	A	.	.	.	.	.	1.22	-0.49	.	*	F	0.60	3.22
Ala	331	.	A	.	.	.	.	C	1.52	-1.27	.	*	F	1.10	3.64
Pro	332	A	A	.	.	.	.	.	1.92	-1.17	*	*	F	0.90	2.13
Glu	333	A	A	.	.	.	.	.	1.62	-1.19	*	*	F	0.90	1.64
Arg	334	A	A	.	.	.	.	.	0.77	-0.80	*	.	F	0.90	2.18
Ala	335	A	A	.	B	.	.	.	0.52	-0.66	*	*	F	0.90	1.05
Ser	336	.	A	B	B	.	.	.	0.80	-0.33	*	*	F	0.45	0.95

Table 1 (continued)

Res	Position	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
Ser	337	.	.	B	B	.	.	.	1.12	0.16	*	.	F	-0.15	0.70
Val	338	.	.	B	B	.	.	.	0.82	0.16	*	*	.	-0.15	1.35
Tyr	339	.	.	B	B	.	.	.	0.40	0.04	*	*	F	0.30	1.35
Thr	340	.	.	B	B	.	.	.	0.64	0.14	*	*	F	0.60	1.46
Arg	341	.	.	.	B	.	.	C	0.94	0.19	.	*	F	1.10	1.94
Ser	342	.	.	.	.	.	T	C	1.24	-0.46	.	*	F	2.40	2.15
Thr	343	.	.	.	.	.	T	C	2.10	-0.81	.	*	F	3.00	2.58
Gly	344	.	.	.	.	.	T	C	1.46	-1.30	.	*	F	2.70	2.28
Glu	345	.	.	.	.	.	T	C	1.47	-0.61	.	*	F	2.40	1.19
Gln	346	.	.	B	B	.	.	.	0.50	-0.61	.	*	F	1.50	1.11
Glu	347	.	.	B	B	.	.	.	0.46	-0.46	.	*	F	0.75	0.83
Ile	348	.	.	B	B	.	.	.	-0.04	-0.46	.	*	F	0.45	0.47
Ser	349	.	.	B	B	.	.	.	-0.09	0.23	.	*	.	-0.30	0.23
Val	350	.	.	B	B	.	.	.	-0.48	0.26	.	*	.	-0.30	0.17
Gly	351	.	.	B	B	.	.	.	-0.87	0.69	.	*	.	-0.60	0.30
Leu	352	A	.	.	B	.	.	.	-1.26	0.43	.	*	.	-0.60	0.29

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Among highly preferred fragments in this regard are those that comprise regions of G-protein Chemokine Receptor (CCR5) that combine several structural features, such as several of the features set out above.

Other preferred polypeptide fragments are biologically active G-protein Chemokine Receptor (CCR5) fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the G-protein Chemokine Receptor (CCR5) polypeptide. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

However, many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO:1 or to the deposited clone and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1400 of SEQ ID NO:1, b is an integer of 15 to 1414, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:1 or of the deposited clone, and where the b is greater than or equal to a + 14.

Epitopes and Antibodies

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide having an amino acid sequence of SEQ ID NO:2, or an epitope of the polypeptide sequence encoded by a polynucleotide sequence contained in ATCC Deposit No: 97183 or encoded by a polynucleotide that hybridizes to the complement of the sequence of SEQ ID NO:1 or contained in ATCC Deposit No: 97183 under stringent hybridization conditions or lower stringency hybridization conditions as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:1 or the sequence of the

deposited clone), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand under stringent hybridization conditions or lower stringency hybridization conditions defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody binds via its antigen binding region (i.e., domains containing the complementarity determining regions or antigen binding site) as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic. Either the full-length protein or an antigenic peptide fragment can be used. Regions having a high antigenicity index are shown in Table 1 and FIG. 3.

Antibodies are preferably prepared from these regions or from discrete fragments in these regions. However, antibodies can be prepared from any region of the peptide as described herein. A preferred fragment produces an antibody that diminishes or completely prevents ligand binding. Antibodies can be developed against the entire receptor or portions of the receptor, for example, the intracellular carboxy terminal domain, the amino terminal extracellular domain, the entire transmembrane domain or specific transmembrane segments, any of the intracellular or extracellular loops, or any portions of these regions. Antibodies may also be developed against specific functional sites, such as the site of ligand binding, the site of G-protein coupling, or sites that are glycosylated, phosphorylated, myristoylated, or amidated.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985), further described in U.S. Patent No. 4,631,211).

In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)). These fragments are not to be construed, however, as encompassing any fragments which may be disclosed prior to the invention.

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985)). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., *J. Gen. Virol.*, 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde.

Epitope bearing peptides of the invention may also be synthesized as multiple antigen peptides (MAPs), first described by J. P. Tam in *Proc. Natl. Acad. Sci. U.S.A.* 85:5409 which is incorporated by reference herein in its entirety. MAPs consist of multiple copies of a specific peptide attached to a non-immunogenic lysine core. Map peptides usually contain four or eight copies of the peptide often referred to as MAP-4 or MAP-8 peptides. By way of non-limiting example, MAPs may be synthesized onto a lysine core matrix attached to a polyethylene glycol-polystyrene (PEG-PS) support. The peptide of interest is synthesized onto the lysine residues using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry. For example, Applied Biosystems (Foster City, CA) offers MAP resins, such as, for example, the Fmoc Resin 4 Branch and the Fmoc Resin 8 Branch which can be used to synthesize MAPs. Cleavage of MAPs from the resin is performed with standard trifluoroacetic acid (TFA)-based cocktails known in the art. Purification of MAPs, except for desalting, is not necessary. MAP peptides may be used as an immunizing vaccine which elicits antibodies that recognize both the MAP and the native protein from which the peptide was derived.

Epitope bearing peptides of the invention may also be incorporated into a coat protein of a virus which can then be used as an immunogen or a vaccine with which to immunize animals, including humans, in order encourage the production of anti-epitope antibodies. For example, the V3 loop of the gp120 glycoprotein of the human immunodeficiency virus type 1 (HIV-1) has been engineered to be expressed on the surface of rhinovirus. Immunization with this rhinovirus displaying the V3 loop peptide

yielded apparently effective mimics of the HIV-1 immunogens (as measured by their ability to be neutralized by anti-HIV-1 antibodies as well as their ability to elicit the production of antibodies capable of neutralizing HIV-1 in cell culture). This techniques of using engineered viral particles as an immunogen is described in more detail in Smith et al., Behring Inst Mitt (98):229-39 (1997), Smith et al, J Virol 72:651-9 (1998), and Zhang et al., Biol Chem 380:365-74 (1999), which are hereby incorporated by reference herein in their entirety.

Epitope bearing polypeptides of the invention may be modified, for example, by the addition of amino acids at the amino- and/or carboxy- termini of the peptide. Such modifications may be performed, for example, to alter the conformation of the epitope bearing polypeptide such that the epitope will have a conformation more closely related to the structure of the epitope in the native protein. An example of a modified epitope-bearing polypeptide of the invention is a polypeptide in which one or more cysteine residues have been added to the polypeptide to allow for the formation of a disulfide bond between two cysteines, resulting in a stable loop structure of the epitope bearing polypeptide under non-reducing conditions. Disulfide bonds may form between a cysteine residue added to the polypeptide and a cysteine residue of the naturally occurring epitope, or may form between two cysteines which have both been added to the naturally occurring epitope bearing polypeptide. Additionally, it is possible to modify one or more amino acid residues of the naturally occurring epitope bearing polypeptide by substituting them with cysteines to promote the formation of disulfide bonded loop structures. Cyclic thioether molecules of synthetic peptides may be routinely generated using techniques known in the art and are described in PCT publication WO 97/46251, incorporated in its entirety by reference herein. Other modifications of epitope-bearing polypeptides contemplated by this invention include biotinylation.

Animals such as rabbits, rats and mice are immunized with either free or carrier-coupled peptides or MAP peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA

assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention comprising an immunogenic or antigenic epitope can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) or albumin (including but not limited to recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., *Nature*, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion disulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., *J. Biochem.*, 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972- 897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a

matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opin. Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:1 and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:2 or of the polypeptide encoded by the deposited clone, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding).

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. *See generally, Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). The variable regions of each light/heavy chain pair form the antibody binding site.

Thus, an intact IgG antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same.

The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hyper variable regions, also called complementarity determining regions or CDRs. The CDRs from the heavy and the light chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989).

A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. *See, e.g.,* Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. *J Immunol.* 148:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al. "Diabodies: small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new

molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)).

The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions or fragments of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. As such the term "antibody" encompasses not only whole antibody molecules, but also antibody multimers and antibody fragments and/or variants (including derivatives) of antibodies, antibody multimers and antibody fragments. Examples of molecules which are described by the term "antibody" herein include, but are not limited to: single chain Fvs (scFvs), Fab fragments, Fab' fragments, F(ab')₂, disulfide linked Fvs (sdFvs), Fvs, and fragments comprising or alternatively consisting of, either a VL or a VH domain. The term "single chain Fv" or "scFv" as used herein refers to a polypeptide comprising a VL domain of antibody linked to a VH domain of an antibody.

Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), intracellularly-made antibodies (i.e., intrabodies), and epitope-binding fragments of any of the above. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In a preferred embodiment, the immunoglobulin is an IgM isotype. In a preferred embodiment, the immunoglobulin is an IgG1 isotype. In another preferred embodiment, the immunoglobulin is an IgG2 isotype. In another preferred embodiment, the immunoglobulin is an IgG4 isotype. Immunoglobulins may have both a heavy and light chain. An array of IgG, IgE, IgM, IgD, IgA, and IgY heavy chains may be paired with a light chain of the kappa or lambda forms.

Antibodies of the invention may also include multimeric forms of antibodies. For example, antibodies of the invention may take the form of antibody dimers, trimers, or higher-order multimers of monomeric immunoglobulin molecules. Dimers of whole immunoglobulin molecules or of F(ab')₂ fragments are tetravalent, whereas dimers of Fab fragments or scFv molecules are bivalent. Individual monomers within an antibody

multimer may be identical or different, i.e., they may be heteromeric or homomeric antibody multimers. For example, individual antibodies within a multimer may have the same or different binding specificities.

Multimerization of antibodies may be accomplished through natural aggregation of antibodies or through chemical or recombinant linking techniques known in the art. For example, some percentage of purified antibody preparations (e.g., purified IgG1 molecules) spontaneously form protein aggregates containing antibody homodimers, and other higher-order antibody multimers. Alternatively, antibody homodimers may be formed through chemical linkage techniques known in the art. For example, heterobifunctional crosslinking agents including, but not limited to, SMCC [succinimidyl 4-(maleimidomethyl)cyclohexane-1-carboxylate] and SATA [N-succinimidyl S-acetylthio-acetate] (available, for example, from Pierce Biotechnology, Inc. (Rockford, IL)) can be used to form antibody multimers. An exemplary protocol for the formation of antibody homodimers is given in Ghetie et al., *Proceedings of the National Academy of Sciences USA* (1997) 94:7509-7514, which is hereby incorporated by reference in its entirety. Antibody homodimers can be converted to Fab'2 homodimers through digestion with pepsin. Another way to form antibody homodimers is through the use of the autophilic T15 peptide described in Zhao and Kohler, *The Journal of Immunology* (2002) 25:396-404, which is hereby incorporated by reference in its entirety.

Alternatively, antibodies can be made to multimerize through recombinant DNA techniques. IgM and IgA naturally form antibody multimers through the interaction with the J chain polypeptide. Non-IgA or non-IgM molecules, such as IgG molecules, can be engineered to contain the J chain interaction domain of IgA or IgM, thereby conferring the ability to form higher order multimers on the non-IgA or non-IgM molecules. (see, for example, Chintalacharuvu et al., (2001) *Clinical Immunology* 101:21-31. and Frigerio et al., (2000) *Plant Physiology* 123:1483-94., both of which are hereby incorporated by reference in their entireties.) ScFv dimers can also be formed through recombinant techniques known in the art; an example of the construction of scFv dimers is given in Goel et al., (2000) *Cancer Research* 60:6964-6971 which is hereby incorporated by reference in its entirety. Antibody multimers may be purified using any suitable method known in the art, including, but not limited to, size exclusion chromatography.

By "isolated antibody" is intended an antibody removed from its native environment. Thus, an antibody produced by, purified from and/or contained within a hybridoma and/or a recombinant host cell is considered isolated for purposes of the present invention.

Unless otherwise defined in the specification, specific binding or immunospecific binding by an anti-CCR5 antibody means that the anti-CCR5 antibody binds CCR5 but does not significantly bind to (i.e., cross react with) proteins other than CCR5, such as other proteins in the same family of proteins). An antibody that binds CCR5 protein and does not cross-react with other proteins is not necessarily an antibody that does not bind said other proteins in all conditions; rather, the CCR5-specific antibody of the invention preferentially binds CCR5 compared to its ability to bind said other proteins such that it will be suitable for use in at least one type of assay or treatment, i.e., give low background levels or result in no unreasonable adverse effects in treatment. It is well known that the portion of a protein bound by an antibody is known as the epitope. An epitope may either be linear (i.e., comprised of sequential amino acids residues in a protein sequences) or conformational (i.e., comprised of one or more amino acid residues that are not contiguous in the primary structure of the protein but that are brought together by the secondary, tertiary or quaternary structure of a protein). Given that CCR5-specific antibodies bind to epitopes of CCR5, an antibody that specifically binds CCR5 may or may not bind fragments of CCR5 and/or variants of CCR5 (e.g., proteins that are at least 90% identical to CCR5) depending on the presence or absence of the epitope bound by a given CCR5-specific antibody in the CCR5 fragment or variant. Likewise, CCR5-specific antibodies of the invention may bind species orthologues of CCR5 (including fragments thereof) depending on the presence or absence of the epitope recognized by the antibody in the orthologue. Additionally, CCR5-specific antibodies of the invention may bind modified forms of CCR5, for example, CCR5 fusion proteins. In such a case when antibodies of the invention bind CCR5 fusion proteins, the antibody must make binding contact with the CCR5 moiety of the fusion protein in order for the binding to be specific. Antibodies that specifically bind to CCR5 can be identified, for example, by immunoassays or other techniques known to those of skill in the art, e.g., the immunoassays described in the Examples below.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')₂, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described *infra* and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, by size in contiguous amino acid residues, or listed in the Tables and Figures. Preferred epitopes of the invention include: Thr16-Val25, Gln59-Thr65, Thr167-Leu174, Ser 179-Ser185, Leu222-Ala233, Asn268-Gln277, His315-Ser325, Glu330-Ser336, Tyr339-Ile348 of SEQ ID NO:2 or of the

polypeptide encoded by the deposited clone, as well as polynucleotides that encode these epitopes. Even more preferred epitopes of the invention include peptides corresponding the extracellular loops of the G-protein Chemokine Receptor (CCR5) of the invention or fragments and variants thereof, e.g., amino acids 89-102, 167-195 and/or 261-274 of SEQ ID NO:2 or of the polypeptide encoded by the deposited clone. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, monkey, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein).

The antibodies of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) may bind immunospecifically to that immunospecifically bind to a polypeptide or polypeptide fragment or variant of human G-protein Chemokine Receptor (CCR5) (SEQ ID NO:2 or of the polypeptide encoded by the

deposited clone) and/or monkey G-protein Chemokine Receptor (CCR5). Preferably, the antibodies of the invention bind immunospecifically to human G-protein Chemokine Receptor. Preferably, the antibodies of the invention bind immunospecifically to human and monkey G-protein Chemokine Receptor. Also preferably, the antibodies of the invention bind immunospecifically to human G-protein Chemokine Receptor (CCR5) and murine G-protein Chemokine Receptor. More preferably, antibodies of the invention, bind immunospecifically and with higher affinity to human G-protein Chemokine Receptor (CCR5) than to murine G-protein Chemokine Receptor.

In preferred embodiments, the antibodies of the present invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), immunospecifically bind to G-protein Chemokine Receptor (CCR5) and do not cross-react with any other antigens. In preferred embodiments, the antibodies of the invention immunospecifically bind to G-protein Chemokine Receptor (CCR5) and do not cross-react with other chemokine receptors such as, for example, US28, CCR1, CCR2, CCR3, CCR4, CCR6, CCR7, CCR8, CCR9, CXCR1, CXCR2, CXCR3, CXCR4, and/or CXCR5.

In other preferred embodiments, the antibodies of the invention immunospecifically bind to G-protein Chemokine Receptor (CCR5) and cross-react with other chemokine receptors such as, for example, US28, CCR1, CCR2, CCR3, CCR4, CCR6, CCR7, CCR8, CCR9, CXCR1, CXCR2, CXCR3, CXCR4, and/or CXCR5. In more preferred embodiments, the antibodies of the invention immunospecifically bind to G-protein Chemokine Receptor (CCR5) and do cross-react with CCR3 and/or CXCR4.

In a preferred embodiment, antibodies of the invention preferentially bind G-protein Chemokine Receptor (CCR5) (SEQ ID NO:2 or of the polypeptide encoded by the deposited clone), or fragments and variants thereof relative to their ability to bind other antigens, (such as, for example, other chemokine receptors).

By way of non-limiting example, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with a dissociation constant (K_D) that is less than the antibody's K_D for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity that is at least one order of magnitude less than the antibody's K_D

for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity that is at least two orders of magnitude less than the antibody's K_D for the second antigen.

In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an off rate (k_{off}) that is less than the antibody's k_{off} for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity that is at least one order of magnitude less than the antibody's k_{off} for the second antigen. In another non-limiting embodiment, an antibody may be considered to bind a first antigen preferentially if it binds said first antigen with an affinity that is at least two orders of magnitude less than the antibody's k_{off} for the second antigen.

Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M. More preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M or 10^{-8} M. Even more preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

In specific embodiments, antibodies of the invention bind G-protein Chemokine Receptor (CCR5) polypeptides or fragments or variants thereof with an off rate (k_{off}) of less than or equal to $5 \times 10^{-2} \text{ sec}^{-1}$, 10^{-2} sec^{-1} , $5 \times 10^{-3} \text{ sec}^{-1}$ or 10^{-3} sec^{-1} . More preferably, antibodies of the invention bind G-protein Chemokine Receptor (CCR5) polypeptides or fragments or variants thereof with an off rate (k_{off}) less than or equal to $5 \times 10^{-4} \text{ sec}^{-1}$, 10^{-4} sec^{-1} , $5 \times 10^{-5} \text{ sec}^{-1}$, or 10^{-5} sec^{-1} , $5 \times 10^{-6} \text{ sec}^{-1}$, 10^{-6} sec^{-1} , $5 \times 10^{-7} \text{ sec}^{-1}$ or 10^{-7} sec^{-1} .

In other embodiments, antibodies of the invention bind G-protein Chemokine Receptor (CCR5) polypeptides or fragments or variants thereof with an on rate (k_{on}) of greater than or equal to $10^3 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^3 \text{ M}^{-1} \text{ sec}^{-1}$, $10^4 \text{ M}^{-1} \text{ sec}^{-1}$ or $5 \times 10^4 \text{ M}^{-1} \text{ sec}^{-1}$. More preferably, antibodies of the invention bind G-protein Chemokine Receptor (CCR5) polypeptides or fragments or variants thereof with an on rate (k_{on}) greater than or equal to $10^5 \text{ M}^{-1} \text{ sec}^{-1}$, $5 \times 10^5 \text{ M}^{-1} \text{ sec}^{-1}$, $10^6 \text{ M}^{-1} \text{ sec}^{-1}$, or $5 \times 10^6 \text{ M}^{-1} \text{ sec}^{-1}$ or $10^7 \text{ M}^{-1} \text{ sec}^{-1}$.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be

specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., *Blood* 92(6):1981-1988 (1998); Chen et al., *Cancer Res.* 58(16):3668-3678 (1998); Harrop et al., *J. Immunol.* 161(4):1786-1794 (1998); Zhu et al., *Cancer Res.* 58(15):3209-3214 (1998); Yoon et al., *J. Immunol.* 160(7):3170-3179 (1998); Prat et al., *J. Cell. Sci.* 111(Pt2):237-247 (1998); Pitard et al., *J. Immunol. Methods* 205(2):177-190 (1997); Liautard et al., *Cytokine* 9(4):233-241 (1997); Carlson et al., *J. Biol. Chem.* 272(17):11295-11301 (1997); Taryman et al., *Neuron* 14(4):755-762 (1995); Muller et al., *Structure* 6(9):1153-1167 (1998); Bartunek et al., *Cytokine* 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

In one embodiment of the present invention, antibodies that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprise a polypeptide having the amino acid sequence of any one of the heavy chains expressed by an anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention and/or any one of the light chains expressed by an anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention. In another embodiment of the present invention, antibodies that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprise a polypeptide having the amino acid sequence of any one of the VH domains of a heavy chain expressed by an anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention and/or any one of the VL domains of a light chain expressed by an anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention. In preferred embodiments, antibodies of the present invention comprise the amino acid sequence of a VH domain and VL domain expressed by a single anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention. In alternative embodiments, antibodies of the present invention comprise the amino acid sequence of a VH domain and a VL domain expressed by two different anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell lines of the invention. Molecules comprising,

or alternatively consisting of, antibody fragments or variants of the VH and/or VL domains expressed by an anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) are also encompassed by the invention, as are nucleic acid molecules encoding these VH and VL domains, molecules, fragments and/or variants.

The present invention also provides antibodies that immunospecifically bind to a polypeptide, or polypeptide fragment or variant of a G-protein Chemokine Receptor (CCR5), wherein said antibodies comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one, two, three, or more of the VH CDRs contained in a heavy chain expressed by one or more anti-G-protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. In particular, the invention provides antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprising, or alternatively consisting of, a polypeptide having the amino acid sequence of a VH CDR1 contained in a heavy chain expressed by one or more anti-G-protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. In another embodiment, antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a VH CDR2 contained in a heavy chain expressed by one or more anti-G-protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. In a preferred embodiment, antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprise, or alternatively consist of a polypeptide having the amino acid sequence of a VH CDR3 contained in a heavy chain expressed by one or more anti-G-protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. Molecules comprising, or alternatively consisting of, these antibodies, or antibody fragments or variants thereof, that immunospecifically bind to G-protein Chemokine Receptor (CCR5) or a G-protein Chemokine Receptor (CCR5) fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments and/or variants.

The present invention also provides antibodies that immunospecifically bind to a polypeptide, or polypeptide fragment or variant of a G-protein Chemokine Receptor (CCR5), wherein said antibodies comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one, two, three, or more of the VL CDRs contained in a light chain expressed by one or more anti-G-protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. In particular, the invention provides antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprising, or alternatively consisting of, a polypeptide having the amino acid sequence of a VL CDR1 contained in a light chain expressed by one or more anti-G-protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. In another embodiment, antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a VL CDR2 contained in a light chain expressed by one or more anti-G-protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. In a preferred embodiment, antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprise, or alternatively consist of a polypeptide having the amino acid sequence of a VL CDR3 contained in a light chain expressed by one or more anti-G-protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. Molecules comprising, or alternatively consisting of, these antibodies, or antibody fragments or variants thereof, that immunospecifically bind to G-protein Chemokine Receptor (CCR5) or a G-protein Chemokine Receptor (CCR5) fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments and/or variants.

The present invention also provides antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants) that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) polypeptide or polypeptide fragment or variant of a G-protein Chemokine Receptor (CCR5), wherein said antibodies comprise, or alternatively consist of, one, two, three, or more VH CDRs and one, two, three or more VL CDRs, as contained in a heavy chain or light chain expressed by one or more anti-G-

protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. In particular, the invention provides for antibodies that immunospecifically bind to a polypeptide or polypeptide fragment or variant of a G-protein Chemokine Receptor (CCR5), wherein said antibodies comprise, or alternatively consist of, a VH CDR1 and a VL CDR1, a VH CDR1 and a VL CDR2, a VH CDR1 and a VL CDR3, a VH CDR2 and a VL CDR1, VH CDR2 and VL CDR2, a VH CDR2 and a VL CDR3, a VH CDR3 and a VH CDR1, a VH CDR3 and a VL CDR2, a VH CDR3 and a VL CDR3, or any combination thereof, of the VH CDRs and VL CDRs contained in a light chain or light chain expressed by one or more anti-G-protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. In a preferred embodiment, one or more of these combinations are from a single anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention. Molecules comprising, or alternatively consisting of, fragments or variants of these antibodies, that immunospecifically bind to G-protein Chemokine Receptor (CCR5) are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments or variants.

The present invention also provides for nucleic acid molecules, generally isolated, encoding an antibody of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof). In a specific embodiment, a nucleic acid molecule of the invention encodes an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), comprising, or alternatively consisting of, a VH domain having an amino acid sequence of any one of the VH domains of a heavy chain expressed by an anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention and a VL domain having an amino acid sequence of a light chain expressed by an anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention. In another embodiment, a nucleic acid molecule of the invention encodes an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), comprising, or alternatively consisting of, a VH domain having an amino acid sequence of any one of the VH domains of a heavy chain expressed by an anti-G-protein Chemokine Receptor(CCR5)

antibody expressing cell line of the invention or a VL domain having an amino acid sequence of a light chain expressed by an anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention.

The present invention also provides antibodies that comprise, or alternatively consist of, variants (including derivatives) of the antibody molecules (e.g., the VH domains and/or VL domains) described herein, which antibodies immunospecifically bind to a G-protein Chemokine Receptor (CCR5) or fragment or variant thereof. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH domain, VHCDR1, VHCDR2, VHCDR3, VL domain, VLCDR1, VLCDR2, or VLCDR3. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (e.g., the ability to bind a G-protein Chemokine Receptor).

For example, it is possible to introduce mutations only in framework regions or only in CDR regions of an antibody molecule. Introduced mutations may be silent or neutral missense mutations, i.e., have no, or little, effect on an antibody's ability to bind antigen. These types of mutations may be useful to optimize codon usage, or improve a hybridoma's antibody production. Alternatively, non-neutral missense mutations may alter an antibody's ability to bind antigen. The location of most silent and neutral missense mutations is likely to be in the framework regions, while the location of most non-neutral missense mutations is likely to be in CDR, though this is not an absolute requirement. One of skill in the art would be able to design and test mutant molecules with desired properties such as no alteration in antigen binding activity or alteration in binding activity (e.g., improvements in antigen binding activity or change in antibody specificity). Following mutagenesis, the encoded protein may routinely be expressed and the functional and/or biological activity of the encoded protein, (e.g., ability to immunospecifically bind a G-protein Chemokine Receptor) can be determined using techniques described herein or by routinely modifying techniques known in the art.

In a specific embodiment, an antibody of the invention (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds G-protein Chemokine Receptor (CCR5) polypeptides or fragments or variants thereof, comprises, or alternatively consists of, an amino acid sequence encoded by a nucleotide sequence that hybridizes to a nucleotide sequence that is complementary to that encoding one of the VH or VL domains expressed by one or more anti-G-protein Chemokine Receptor (CCR5) antibody expressing cell lines of the invention. under stringent conditions, e.g., hybridization to filter-bound DNA in 6X sodium chloride/sodium citrate (SSC) at about 45° C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65° C, under highly stringent conditions, e.g., hybridization to filter-bound nucleic acid in 6xSSC at about 45° C followed by one or more washes in 0.1xSSC/0.2% SDS at about 68° C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds. , 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing

Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3). Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

It is well known within the art that polypeptides, or fragments or variants thereof, with similar amino acid sequences often have similar structure and many of the same biological activities. Thus, in one embodiment, an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds to a G-protein Chemokine Receptor (CCR5) polypeptide or fragments or variants of a G-protein Chemokine Receptor (CCR5) polypeptide, comprises, or alternatively consists of, a VH domain having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to the amino acid sequence of a VH domain of a heavy chain expressed by an anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention.

In another embodiment, an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds to a G-protein Chemokine Receptor (CCR5) polypeptide or fragments or variants of a G-protein Chemokine Receptor (CCR5) polypeptide, comprises, or alternatively consists of, a VL domain having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to the amino acid sequence of a VL domain of a light chain expressed by an anti-G-protein Chemokine Receptor(CCR5) antibody expressing cell line of the invention.

The invention also encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that have one or more of the same biological characteristics as one or more of the antibodies described herein. By "biological characteristics" is meant, the in vitro or in vivo activities or properties of the antibodies, such as, for example, the ability to bind to G-protein Chemokine Receptor (CCR5) (e.g., G-protein Chemokine Receptor (CCR5) expressed on a cell surface, such as

in Example 67, membrane-embedded G-protein Chemokine Receptor (CCR5), and/or a fragment or variant of G-protein Chemokine Receptor (CCR5)); the ability to substantially inhibit or abolish the binding of the G-protein Chemokine Receptor (CCR5) to a G-protein Chemokine Receptor (CCR5) ligand (e.g. MIP1-beta, see, e.g., Example 61); the ability to downregulate G-protein Chemokine Receptor (CCR5) expression on the surface of cells; the ability to inhibit or abolish G-protein Chemokine Receptor (CCR5) mediated biological activity (e.g., HIV binding to, infection (entry into/fusion), and/or replication in, G-protein Chemokine Receptor (CCR5) expressing cells (see, e.g., Examples 53, 60, 68 and 69), the ability to inhibit or abolish MIP1-beta induced chemotaxis of peripheral blood mononuclear cells PBMC (or other G-protein Chemokine Receptor (CCR5) expressing cells), or the ability to induce an intracellular calcium flux in G-protein Chemokine Receptor (CCR5) expressing cells, (see, e.g., Example 63). Optionally, the antibodies of the invention will bind to the same epitope as at least one of the antibodies specifically referred to herein. Such epitope binding can be routinely determined using assays known in the art.

The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that neutralize G-protein Chemokine Receptor (CCR5), said antibodies comprising, or alternatively consisting of, a portion (e.g., VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3) of a VH or VL domain of an antibody of the invention. An antibody that "neutralizes G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof" is, for example, an antibody that diminishes or abolishes the ability of G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof to bind to its ligand (e.g., HIV and MIP1-beta); that diminishes or abolishes MIP1-beta induced chemotaxis of PBMC or other CCR5 expressing cell; and/or that abolishes or inhibits the G-protein Chemokine Receptor (CCR5) signaling cascade (e.g., calcium flux initiated by an activated G-protein Chemokine Receptor (CCR5), see, e.g., Example 63). In one embodiment, an antibody that neutralizes G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain of an antibody of the invention, or a fragment or variant thereof and a VL domain of an antibody of the invention, or a fragment or variant thereof. In another

embodiment, an antibody that neutralizes G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain and a VL domain from a single antibody (or scFv or Fab fragment) of the invention, or fragments or variants thereof. In one embodiment, an antibody that neutralizes G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that neutralizes G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that neutralizes G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH CDR domain of an antibody of the invention, or a fragment or variant thereof. In a preferred embodiment, an antibody that neutralizes G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH CDR3 of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that neutralizes G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL CDR of an antibody of the invention, or a fragment or variant thereof. In another preferred embodiment, an antibody that neutralizes G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL CDR3 of an antibody of the invention, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that reduces or abolishes the ability of HIV viruses, particularly those that utilize G-protein Chemokine Receptor (CCR5) as a co-receptor, to bind to, infect (enter into/fuse with), and/or replicate in G-protein Chemokine Receptor (CCR5) expressing cells, as determined by any method known in the art such as, for example, the assays described Examples 60,

68 and 69. Said antibodies may comprise, or alternatively consist of, a portion (*e.g.*, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3) of a VH or VL domain having an amino acid sequence of an antibody of the invention or a fragment or variant thereof. In one embodiment, an antibody that reduces or abolishes the ability of HIV viruses, particularly those that utilize G-protein Chemokine Receptor (CCR5) as a co-receptor, to bind to, infect (enter into/fuse with), and/or replicate in G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain of an antibody of the invention, or a fragment or variant thereof and a VL domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that reduces or abolishes the ability of HIV viruses, particularly those that utilize G-protein Chemokine Receptor (CCR5) as a co-receptor, to bind to, infect (enter into/fuse with), and/or replicate in G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain and a VL domain from a single antibody (or scFv or Fab fragment) of the invention, or fragments or variants thereof. In one embodiment, an antibody that reduces or abolishes the ability of HIV viruses, particularly those that utilize G-protein Chemokine Receptor (CCR5) as a co-receptor, to bind to, infect (enter into/fuse with), and/or replicate in G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that reduces or abolishes the ability of HIV viruses, particularly those that utilize G-protein Chemokine Receptor (CCR5) as a co-receptor, to bind to, infect (enter into/fuse with), and/or replicate in G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL domain of an antibody of the invention, or a fragment or variant thereof. In a preferred embodiment, an antibody that reduces or abolishes the ability of HIV viruses, particularly those that utilize G-protein Chemokine Receptor (CCR5) as a co-receptor, to bind to, infect (enter into/fuse with), and/or replicate in G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH CDR3 of an antibody of the invention, or a fragment or variant thereof. In another preferred

embodiment, an antibody that reduces or abolishes the ability of HIV viruses, particularly those that utilize G-protein Chemokine Receptor (CCR5) as a co-receptor, to bind to, infect (enter into/fuse with), and/or replicate in G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL CDR3 of an antibody of the invention, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that inhibits or abolishes MIP1-beta induced chemotaxis of peripheral blood mononuclear cells PBMC or other G-protein Chemokine Receptor (CCR5) expressing cells, as determined by any method known in the art such as, for example, the assays described in Example 62. Said antibodies may comprise, or alternatively consist of, a portion (*e.g.*, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3) of a VH or VL domain having an amino acid sequence of an antibody of the invention or a fragment or variant thereof. In one embodiment, an antibody that inhibits or abolishes MIP1-beta induced chemotaxis of peripheral blood mononuclear cells PBMC or other G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain of an antibody of the invention, or a fragment or variant thereof and a VL domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that inhibits or abolishes MIP1-beta induced chemotaxis of peripheral blood mononuclear cells PBMC or other G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain and a VL domain from a single antibody (or scFv or Fab fragment) of the invention, or fragments or variants thereof. In one embodiment, an antibody that inhibits or abolishes MIP1-beta induced chemotaxis of peripheral blood mononuclear cells PBMC or other G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that inhibits or abolishes MIP1-beta induced chemotaxis of peripheral blood mononuclear cells PBMC or other G-protein

Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL domain of an antibody of the invention, or a fragment or variant thereof. In a preferred embodiment, an antibody that inhibits or abolishes MIP1-beta induced chemotaxis of peripheral blood mononuclear cells PBMC or other G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH CDR3 of an antibody of the invention, or a fragment or variant thereof. In another preferred embodiment, an antibody that inhibits or abolishes MIP1-beta induced chemotaxis of peripheral blood mononuclear cells PBMC or other G-protein Chemokine Receptor (CCR5) expressing cells, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL CDR3 of an antibody of the invention, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that downregulates the cell-surface expression of G-protein Chemokine Receptor (CCR5), as determined by any method known in the art such as, for example, FACS analysis/the assays described in Examples 61 or 63. By way of a non-limiting hypothesis, such down regulation may be the result of antibody induced internalization of G-protein Chemokine Receptor (CCR5). Said antibodies may comprise, or alternatively consist of, a portion (*e.g.*, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3) of a VH or VL domain having an amino acid sequence of an antibody of the invention or a fragment or variant thereof. In one embodiment, an antibody that downregulates the cell-surface expression of G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain of an antibody of the invention, or a fragment or variant thereof and a VL domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that downregulates the cell-surface expression of G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain and a VL domain from a single antibody (or scFv or Fab fragment) of the invention, or fragments or variants thereof. In one embodiment, an antibody that

downregulates the cell-surface expression of G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that downregulates the cell-surface expression of G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL domain of an antibody of the invention, or a fragment or variant thereof. In a preferred embodiment, an antibody that downregulate the cell-surface expression of G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH CDR3 of an antibody of the invention, or a fragment or variant thereof. In another preferred embodiment, an antibody that downregulates the cell-surface expression of G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL CDR3 of an antibody of the invention, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

The present invention also provides for antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that enhance the activity of G-protein Chemokine Receptor (CCR5), said antibodies comprising, or alternatively consisting of, a portion (*e.g.*, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, or VL CDR3) of a VH or VL domain of an antibody of the invention, or a fragment or variant thereof. By way of non-limiting example, an antibody that “enhances the activity of G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof” is an antibody increases the ability of G-protein Chemokine Receptor (CCR5) to bind to stimulate chemotaxis of PBMC (or other G-protein Chemokine Receptor (CCR5) expressing cells), and/or to stimulate the G-protein Chemokine Receptor (CCR5) signaling cascade (*e.g.*, to initiate an intracellular calcium flux, See Example 63). In one embodiment, an antibody that that enhances the activity of G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain of an antibody of the invention, or a fragment or variant thereof and a VL domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that enhances the activity of G-protein Chemokine Receptor (CCR5), comprises, or alternatively consists of, a polypeptide having the amino

acid sequence of a VH domain and a VL domain from a single antibody (or scFv or Fab fragment) of the invention, or fragments or variants thereof. In one embodiment, an antibody that enhances the activity of G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that enhances the activity of G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL domain of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that enhances the activity of G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH CDR domain referred to in Table 2, or a fragment or variant thereof. In a preferred embodiment, an antibody that enhances the activity of G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VH CDR3 of an antibody of the invention, or a fragment or variant thereof. In another embodiment, an antibody that enhances G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL CDR domain of an antibody of the invention, or a fragment or variant thereof. In another preferred embodiment, an antibody that enhances the activity of G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprises, or alternatively consists of, a polypeptide having the amino acid sequence of a VL CDR3 of an antibody of the invention, or a fragment or variant thereof. Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

The present invention also provides for fusion proteins comprising, or alternatively consisting of, an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), that immunospecifically binds to G-protein Chemokine Receptor (CCR5), and a heterologous polypeptide. Preferably, the heterologous polypeptide to which the antibody is fused to is useful for function or is useful to target the G-protein Chemokine Receptor (CCR5) expressing cells, including but not limited to, MIP-1-beta; a CD4 binding polypeptide such as an anti-CD4 antibody; a

CXCR4 binding polypeptides such as stromal derived factor 1-alpha (SDF1-alpha); and/or a CCR3 binding protein, such as MIP1-alpha). In an alternative preferred embodiment, the heterologous polypeptide to which the antibody is fused to is useful for T cell, macrophage, and/or monocyte cell function or is useful to target the antibody to a T cell, macrophage, or monocyte, including but not limited to, MIP-1-beta; a CD4 binding polypeptide such as an anti-CD4 antibody; a CXCR4 binding polypeptides such as stromal derived factor 1-alpha (SDF1-alpha); and/or a CCR3 binding protein, such as MIP1-alpha). In one embodiment, a fusion protein of the invention comprises, or alternatively consists of, a polypeptide having the amino acid sequence of any one or more of the VH domains of an antibody of the invention or the amino acid sequence of any one or more of the VL domains of an antibody of the invention or fragments or variants thereof, and a heterologous polypeptide sequence. In another embodiment, a fusion protein of the present invention comprises, or alternatively consists of, a polypeptide having the amino acid sequence of any one, two, three, or more of the VH CDRs of an antibody of the invention, or the amino acid sequence of any one, two, three, or more of the VL CDRs of an antibody of the invention, or fragments or variants thereof, and a heterologous polypeptide sequence. In a preferred embodiment, the fusion protein comprises, or alternatively consists of, a polypeptide having the amino acid sequence of, a VH CDR3 of an antibody of the invention, or fragment or variant thereof, and a heterologous polypeptide sequence, which fusion protein immunospecifically binds to G-protein Chemokine Receptor (CCR5). In another embodiment, a fusion protein comprises, or alternatively consists of a polypeptide having the amino acid sequence of at least one VH domain of an antibody of the invention and the amino acid sequence of at least one VL domain of an antibody of the invention or fragments or variants thereof, and a heterologous polypeptide sequence. Preferably, the VH and VL domains of the fusion protein correspond to a single antibody (or scFv or Fab fragment) of the invention. In yet another embodiment, a fusion protein of the invention comprises, or alternatively consists of a polypeptide having the amino acid sequence of any one, two, three or more of the VH CDRs of an antibody of the invention and the amino acid sequence of any one, two, three or more of the VL CDRs of an antibody of the invention, or fragments or variants thereof, and a heterologous polypeptide sequence. Preferably, two, three, four, five, six, or more of

the VHCDR(s) or VLCDR(s) correspond to single antibody (or scFv or Fab fragment) of the invention. Nucleic acid molecules encoding these fusion proteins are also encompassed by the invention.

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

By way of another non-limiting example, antibodies of the invention may be administered to individuals as a form of passive immunization. Alternatively, antibodies of the present invention may be used for epitope mapping to identify the epitope(s) bound by the antibody. Epitopes identified in this way may, in turn, for example, be used as vaccine candidates, i.e., to immunize an individual to elicit antibodies against the naturally occurring forms of G-protein Chemokine Receptor.

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e., by the covalent attachment of any type of molecule to the antibody. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be

carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is

harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 or P3X63-AG8.653 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Another well known method for producing both polyclonal and monoclonal human B cell lines is transformation using Epstein Barr Virus (EBV). Protocols for generating EBV-transformed B cell lines are commonly known in the art, such as, for example, the protocol outlined in Chapter 7.22 of *Current Protocols in Immunology*, Coligan et al., Eds., 1994, John Wiley & Sons, NY, which is hereby incorporated in its entirety by reference herein. The source of B cells for transformation is commonly human peripheral blood, but B cells for transformation may also be derived from other sources including, but not limited to, lymph nodes, tonsil, spleen, tumor tissue, and infected tissues. Tissues are generally made into single cell suspensions prior to EBV transformation. Additionally, steps may be taken to either physically remove or inactivate T cells (e.g., by treatment with cyclosporin A) in B cell-containing samples, because T cells from individuals seropositive for anti-EBV antibodies can suppress B cell immortalization by EBV. In general, the sample containing human B cells is inoculated with EBV, and cultured for 3-4 weeks. A typical source of EBV is the culture supernatant of the B95-8 cell line (ATCC #VR-1492). Physical signs of EBV transformation can generally be seen towards the end of the 3-4 week culture period. By phase-contrast microscopy, transformed cells may appear large, clear, hairy and tend to aggregate in tight clusters of

cells. Initially, EBV lines are generally polyclonal. However, over prolonged periods of cell cultures, EBV lines may become monoclonal or polyclonal as a result of the selective outgrowth of particular B cell clones. Alternatively, polyclonal EBV transformed lines may be subcloned (e.g., by limiting dilution culture) or fused with a suitable fusion partner and plated at limiting dilution to obtain monoclonal B cell lines. Suitable fusion partners for EBV transformed cell lines include mouse myeloma cell lines (e.g., SP2/0, X63-Ag8.653), heteromyeloma cell lines (human x mouse; e.g., SPAM-8, SBC-H20, and CB-F7), and human cell lines (e.g., GM 1500, SKO-007, RPMI 8226, and KR-4). Thus, the present invention also provides a method of generating polyclonal or monoclonal human antibodies against polypeptides of the invention or fragments thereof, comprising EBV-transformation of human B cells.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')₂ fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187

9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., *BioTechniques* 4:214 (1986); Gillies et al., (1989) *J. Immunol. Methods* 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions

will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to

produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; 5,939,598; 6,075,181; and 6,114,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Fremont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., *Bio/technology* 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotypic antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, *FASEB J.* 7(5):437-444; (1989) and Nissinoff, J. *Immunol.* 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-

idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby activate or block its biological activity.

Intrabodies are antibodies, often scFvs, that expressed from a recombinant nucleic acid molecule and engineered to be retained intracellularly (e.g., retained in the cytoplasm, endoplasmic reticulum, or periplasm). Intrabodies may be used, for example, to ablate the function of a protein to which the intrabody binds. The expression of intrabodies may also be regulated through the use of inducible promoters in the nucleic acid expression vector comprising the intrabody. Intrabodies of the invention can be produced using methods known in the art, such as those disclosed and reviewed in Chen *et al.*, *Hum. Gene Ther.* 5:595-601 (1994); Marasco, W.A., *Gene Ther.* 4:11-15 (1997); Rondon and Marasco, *Annu. Rev. Microbiol.* 51:257-283 (1997); Proba *et al.*, *J. Mol. Biol.* 275:245-253 (1998); Cohen *et al.*, *Oncogene* 17:2445-2456 (1998); Ohage and Steipe, *J. Mol. Biol.* 291:1119-1128 (1999); Ohage *et al.*, *J. Mol. Biol.* 291:1129-1134 (1999); Wirtz and Steipe, *Protein Sci.* 8:2245-2250 (1999); Zhu *et al.*, *J. Immunol. Methods* 231:207-222 (1999); and references cited therein. In particular, a CCR5 intrabody has been produced by Steinberger *et al.*, *Proc. Natl. Acad. Sci. USA* 97:805-810 (2000).

XenoMouse Technology

Antibodies in accordance with the invention are preferably prepared by the utilization of a transgenic mouse that has a substantial portion of the human antibody producing genome inserted but that is rendered deficient in the production of endogenous, murine, antibodies (e.g., XenoMouse strains available from Abgenix Inc., Fremont, CA). Such mice, then, are capable of producing human immunoglobulin molecules and antibodies and are deficient in the production of murine immunoglobulin molecules and antibodies. Technologies utilized for achieving the same are disclosed in the patents, applications, and references disclosed herein.

The ability to clone and reconstruct megabase-sized human loci in YACs and to introduce them into the mouse germline provides a powerful approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful

models of human disease. Furthermore, the utilization of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

An important practical application of such a strategy is the "humanization" of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (Mabs) an important milestone towards fulfilling the promise of antibody therapy in human disease.

Fully human antibodies are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized monoclonal antibodies and thus to increase the efficacy and safety of the administered antibodies. The use of fully human antibodies can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as cancer, which require repeated antibody administrations.

One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human Monoclonal antibodies with the desired specificity could be readily produced and selected.

This general strategy was demonstrated in connection with the generation of the first XenoMouse™ strains as published in 1994. See Green et al. Nature Genetics 7:13-21 (1994). The XenoMouse™ strains were engineered with yeast artificial chromosomes

(YACS) containing 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. *Id.* The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes. This was demonstrated by their ability to induce B-cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human monoclonal antibodies. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively, to produce XenoMouse™ mice. See Mendez et al. *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J Exp. Med.* 188:483-495 (1998), Green, *Journal of Immunological Methods* 231:11-23 (1999) and U.S. Patent Application Serial No. 08/759,620, filed December 3, 1996, the disclosures of which are hereby incorporated by reference.

Such approach is further discussed and delineated in U.S. Patent Application Serial Nos. 07/466,008, filed January 12, 1990, 07/710,515, filed November 8, 1990, 07/919,297, filed July 24, 1992, 07/922,649, filed July 30, 1992, filed 08/031,801, filed March 15, 1993, 08/112,848, filed August 27, 1993, 08/234,145, filed April 28, 1994, 08/376,279, filed January 20, 1995, 08/430, 938, April 27, 1995, 08/464,584, filed June 5, 1995, 08/464,582, filed June 5, 1995, 08/471,191, filed June 5, 1995, 08/462,837, filed June 5, 1995, 08/486,853, filed June 5, 1995, 08/486,857, filed June 5, 1995, 08/486,859, filed June 5, 1995, 08/462,513, filed June 5, 1995, 08/724,752, filed October 2, 1996, and 08/759,620, filed December 3, 1996. See also Mendez et al. *Nature Genetics* 15:146-156 (1997) and Green and Jakobovits *J Exp. Med.* 188:483-495 (1998). See also European Patent No., EP 0 471 151 B1, grant published June 12, 1996, International Patent Application No., WO 94/02602, published February 3, 1994, International Patent

Application No., WO 96/34096, published October 31, 1996, and WO 98/24893, published June 11, 1998. The disclosures of each of the above-cited patents, applications, and references are hereby incorporated by reference in their entirety.

Human anti-mouse antibody (HAMA) responses have led the industry to prepare chimeric or otherwise humanized antibodies. While chimeric antibodies have a human constant region and a murine variable region, it is expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody. Thus, it would be desirable to provide fully human antibodies against G-protein Chemokine Receptor (CCR5) polypeptides in order to vitiate concerns and/or effects of HAMA or HACA responses.

Monoclonal antibodies specific for G-protein Chemokine Receptor (CCR5) polypeptides were prepared using hybridoma technology. (Kohler et al., *Nature* 256:495 (1975); Kohler et al., *Eur. J. Immunol.* 6:511 (1976); Kohler et al., *Eur. J. Immunol.* 6:292 (1976); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 571-681 (1981)). Briefly, XenoMouse™ mice were immunized with cells transfected with a G-protein Chemokine Receptor (CCR5) expression vector (for details, see Example 54). After immunization, the splenocytes of such mice were extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (P3X63-AG8.653), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (*Gastroenterology* 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the G-protein Chemokine Receptor (CCR5) polypeptides.

The present invention is directed to fully human antibodies, generally isolated, that immunospecifically bind G-protein Chemokine Receptor (CCR5) polypeptides. Essentially, XenoMouse lines of mice from Abgenix, Inc. (Fremont, CA) expressing human antibodies were immunized with G-protein Chemokine Receptor (CCR5) expressing cells (for details of immunization protocols, see Example 54); spleen and/or lymph node cells (containing B-cells) were recovered from the mice that had high titers of

anti-G-protein Chemokine Receptor (CCR5) antibodies; and such recovered cells were fused with a myeloid-type cell line to prepare immortal hybridoma cell lines. Hybridoma cell lines were screened to select and identify hybridoma cell lines that produced antibodies specific to the immunogen. We utilized these techniques in accordance with the present invention for the preparation of antibodies specific to G-protein Chemokine Receptor (CCR5) polypeptides. Herein, we describe the production of multiple hybridoma cell lines that produce antibodies specific to G-protein Chemokine Receptor (CCR5) polypeptides. Further, we provide a characterization of the antibodies produced by such cell lines.

The antibodies derived from hybridoma cell lines discussed herein are listed in Table 2. Preferred antibodies of the invention include, antibodies expressed by the following cell lines: XF11.1D8, XF11.4D10, XF11.4C4, XF11.5H1, XF11.1G8, XF22.3C9.6, XF22.9E6, XF27/28.7D5, XF27/28.18B5, XF27/28.25G10, XF27/28.36A12, XF27/28.36F11, XF27/28.43E2, and XF27/28.55G4. XenoMouse strains of mice from Abgenix, Inc. express human kappa light chains with either human IgG1, IgG2, or IgG4. Each of the XenoMouse strains can also produce antibodies of the human IgM isotype. The IgG2 expressing strain was used to make the hybridoma cell lines and antibodies of the present invention, thus each of the antibodies produced by hybridoma cell lines are either fully human IgG2 heavy chains with human kappa light chains or IgM heavy chains with human kappa light chains. These hybridoma cell lines were deposited with the American Type Culture Collection ("ATCC") on the date listed in Table 2, and given ATCC Deposit Numbers listed in Table 2. The ATCC is located at 10801 University Boulevard, Manassas, VA 20110-2209, USA. The ATCC deposit was made pursuant to the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for Purposes of Patent Procedure.

Stable cell lines secreting monoclonal antibodies specific for G-protein Chemokine Receptor (CCR5) polypeptides were prepared from certain of the hybridomas described above using recombinant DNA techniques. More specifically, nucleotide sequences encoding the VH and VL domain of the antibody expressed by the XF27/28.36F11 hybridoma (SEQ ID NO:71 (VH) and SEQ ID NO:73 (VL)) were cloned into a vector comprising sequences encoding the human IgG4 (SEQ ID NO:82) and kappa (SEQ ID

NO:83) constant regions. This vector allows for the secretion of an antibody comprising a heavy chain with the VH domain of SEQ ID NO:72 fused to the IgG4 constant domain (SEQ ID NO:82) and a light chain comprising the VL domain of SEQ ID NO:74 fused to the human kappa constant domain (SEQ ID NO:83) by a transfected cell line. Nucleotide sequences encoding the VH and VL domain of the antibody expressed by the XF27/28.43E2 hybridoma (SEQ ID NO:75 (VH) and SEQ ID NO:77 (VL)) were cloned into a vector comprising sequences encoding the human IgG4 (SEQ ID NO:82) and kappa (SEQ ID NO:83) constant regions. This vector allows for the secretion of a antibody comprising a heavy chain with the VH domain of SEQ ID NO:76 fused to the IgG4 constant domain (SEQ ID NO:82) and a light chain comprising the VL domain of SEQ ID NO:78 fused to the human kappa constant domain (SEQ ID NO:83) by a transfected cell line. These vectors were used to stably transfect NS0 cells to produce cell lines NS0mAb36F11 and NS0mAb43E2, respectively.

Polynucleotides encoding the VH and VL domains of the XF27/28.36F11 hybridoma were cloned and used to create a vector encoding an scFv (36F11 scFv) that comprised polynucleotides sequences encoding the VH and VL domains of the antibody produced by the XF27/28.36F11 hybridoma. Variants of the 36F11 scFv expression vector were generated and used to select for affinity matured variants of the 36F11 scFv using phage display technology. One such affinity matured 36F11 scFv variant that was identified is AK38181. A polynucleotide encoding the VL domain of AK38181 (SEQ ID NO:79) was cloned into an expression vector comprising a kappa chain constant domain and polynucleotide sequence encoding the VH domain of either the antibody produced by the XF27/28.36F11 or XF27/28.43E2 cell line, in association with a nucleotide sequence encoding a human IgG4 heavy chain constant domain. These vectors allow for the secretion of an antibody comprising a heavy chain with either the VH domain of SEQ ID NO:72 or 76 fused to the IgG4 constant domain (SEQ ID NO:82) and a light chain comprising the VL domain of SEQ ID NO:80 fused to the human kappa constant domain (SEQ ID NO:83) by a transfected cell line. These vectors were used to stably transfect NS0 cells to produce cell lines NS0mAb36F11/AK38181 and NS0mAb43E2/AK38181, respectively. These cell lines are also listed in Table 2.

XF11.1D8 was deposited at the ATCC on February 7, 2001 and given ATCC Deposit Number PTA-3030. Hybridoma XF11.4D10 was deposited at the ATCC on February 7, 2001 and given ATCC Deposit Number PTA-3026. Hybridoma XF11.4C4 was deposited at the ATCC on February 7, 2001 and given ATCC Deposit Number PTA-3028. Hybridoma XF11.5H1 was deposited at the ATCC on February 7, 2001 and given ATCC Deposit Number PTA-3029. Hybridoma XF11.1G8 was deposited at the ATCC on February 7, 2001 and given ATCC Deposit Number PTA-3027. Hybridoma XF3.5F1 was deposited at the ATCC on March 2, 2001 and given ATCC Deposit Number PTA-3147. Hybridoma XF11.1F8 was deposited at the ATCC on March 2, 2001 and given ATCC Deposit Number PTA-3150. Hybridoma XF3.6A2 was deposited at the ATCC on March 2, 2001 and given ATCC Deposit Number PTA-3148. Hybridoma XF3.10B8 was deposited at the ATCC on March 2, 2001 and given ATCC Deposit Number PTA-3151. Hybridoma XF22.3C9.6 was deposited at the ATCC on September 12, 2001 and given ATCC Deposit Number PTA-3702. Hybridoma XF22.9E6 was deposited at the ATCC on November 14, 2001 and given ATCC Deposit Number PTA-3859. Hybridoma XF27/28.7D5 was deposited at the ATCC on February 1, 2002 and given ATCC Deposit Number PTA-4049. Hybridoma XF27/28.18B5 was deposited at the ATCC on February 1, 2002 and given ATCC Deposit Number PTA-4050. Hybridoma XF27/28.25G10 was deposited at the ATCC on February 1, 2002 and given ATCC Deposit Number PTA-4051. Hybridoma XF27/28.36A12 was deposited at the ATCC on February 1, 2002 and given ATCC Deposit Number PTA-4052. Hybridoma XF27/28.36F11 was deposited at the ATCC on February 1, 2002 and given ATCC Deposit Number PTA-4053. Hybridoma XF27/28.43E2 was deposited at the ATCC on February 1, 2002 and given ATCC Deposit Number PTA-4054. Hybridoma XF27/28.55G4 was deposited at the ATCC on _____ and given ATCC Deposit Number PTA-____. Recombinant cell line NS0mAb36F11 was deposited at the ATCC on _____ and given ATCC Deposit Number _____. Recombinant cell line NS0mAb43E2 was deposited at the ATCC on March 15, 2004 and given ATCC Deposit Number PTA-5860. Recombinant cell line NS0mAb36F11/AK38181 was deposited at the ATCC on March 15, 2004 and given

ATCC Deposit Number PTA-5859. Recombinant cell line NS0mAb43E2/AK38181 was deposited at the ATCC on March 15, 2004 and given ATCC Deposit Number PTA-5861. The ATCC Deposit Numbers and the Hybridoma designations are also presented in Table 2.

Table 2: Cell Lines Expressing anti-G-protein Chemokine Receptor (CCR5) Receptor Antibodies

Cell Line	ATCC Deposit Number	ATCC Deposit Date
XF11.1D8	PTA-3030	February 7, 2001
XF11.4D10	PTA-3026	February 7, 2001
XF11.4C4	PTA-3028	February 7, 2001
XF11.5H1	PTA-3029	February 7, 2001
XF11.1G8	PTA-3027	February 7, 2001
XF3.5F1	PTA-3147	March 2, 2001
XF11.1F8	PTA-3150	March 2, 2001
XF3.6A2	PTA-3148	March 2, 2001
XF3.10B8	PTA-3151	March 2, 2001
XF22.3C9.6	PTA-3702	September 12, 2001
XF22.9E6	PTA-3859	November 14, 2001
XF27/28.7D5	PTA-4049	February 1, 2002
XF27/28.18B5	PTA-4050	February 1, 2002
XF27/28.25G10	PTA-4051	February 1, 2002
XF27/28.36A12	PTA-4052	February 1, 2002
XF27/28.36F11	PTA-4053	February 1, 2002
XF27/28.43E2	PTA-4054	February 1, 2002
XF27/28.55G4		
NS0mAb36F11		
NS0mAb43E2	PTA-5860	March 15, 2004
NS0mAb36F11/AK38181	PTA-5859	March 15, 2004
NS0mAb43E2/AK38181	PTA-5861	March 15, 2004

In one embodiment, the present invention provides hybridoma cell lines expressing an antibody of the invention. In specific embodiments, the hybridoma cell line of the invention is XF11.1D8. In another specific embodiment, the hybridoma cell line of the invention is XF11.4D10. In another specific embodiment, the hybridoma cell line of the invention is XF11.4C4. In another specific embodiment, the hybridoma cell line of the invention is XF11.5H1. In another specific embodiment, the hybridoma cell line of the invention is XF11.1G8. In another specific embodiment, the hybridoma cell line of the invention is XF3.5F1. In another specific embodiment, the hybridoma cell line of the

invention is XF11.1F8. In another specific embodiment, the hybridoma cell line of the invention is XF3.6A2. In another specific embodiment, the hybridoma cell line of the invention is XF3.10B8. In another specific embodiment, the hybridoma cell line of the invention is XF22.3C9.6. In another specific embodiment, the hybridoma cell line of the invention is XF22.9E6. In another specific embodiment, the hybridoma cell line of the invention is XF27/28.7D5. In another specific embodiment, the hybridoma cell line of the invention is XF27/28.18B5. In another specific embodiment, the hybridoma cell line of the invention is XF27/28.25G10. In another specific embodiment, the hybridoma cell line of the invention is XF27/28.36A12. In another specific embodiment, the hybridoma cell line of the invention is XF27/28.36F11. In another specific embodiment, the hybridoma cell line of the invention is XF27/28.43E2. In another specific embodiment, the hybridoma cell line of the invention is XF27/28.55G4. In another specific embodiment, the cell line of the invention is NS0mAb36F11. In another specific embodiment, the cell line of the invention is NS0mAb43E2. In another specific embodiment, the cell line of the invention is NS0mAb36F11/AK38181. In another specific embodiment, the cell line of the invention is NS0mAb43E2/AK38181.

The present invention encompasses antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof) that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) polypeptide or a fragment, variant, or fusion protein thereof. A G-protein Chemokine Receptor (CCR5) polypeptide includes, but is not limited to, the G-protein Chemokine Receptor (CCR5) polypeptide of SEQ ID NO:2 or the polypeptide encoded by the DNA in clone HDGNR10 contained in ATCC Deposit 97183 deposited June 1, 1995; G-protein Chemokine Receptor (CCR5) may be produced through recombinant expression of nucleic acids encoding the polypeptides of SEQ ID NOS:2 or the HDGNR10 DNA in ATCC Deposit Number 97183).

In one embodiment of the present invention, antibodies that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprise a polypeptide having the amino acid sequence of any one of the heavy chains

expressed by at least one of the cell lines referred to in Table 2 and/or any one of the light chains expressed by at least one of the cell lines referred to in Table 2. In another embodiment of the present invention, antibodies that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) or a fragment or variant thereof, comprise a polypeptide having the amino acid sequence of any one of the VH domains of a heavy chain expressed by at least one of the cell lines referred to in Table 2 and/or any one of the VL domains of a light chain expressed by at least one of the cell lines referred to in Table 2. In preferred embodiments, antibodies of the present invention comprise the amino acid sequence of a VH domain and VL domain expressed by the same cell line selected from the group consisting of cell lines referred to in Table 2. In alternative embodiments, antibodies of the present invention comprise the amino acid sequence of a VH domain and a VL domain from different cell lines referred to in Table 2. Molecules comprising, or alternatively consisting of, antibody fragments or variants of the VH and/or VL domains expressed by at least one of the cell lines referred to in Table 2 that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) are also encompassed by the invention, as are nucleic acid molecules encoding these VH and VL domains, molecules, fragments and/or variants.

The present invention also provides antibodies that immunospecifically bind to a polypeptide, or polypeptide fragment or variant of a G-protein Chemokine Receptor (CCR5), wherein said antibodies comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one, two, three, or more of the VH CDRs contained in a heavy chain expressed by one or more cell lines referred to in Table 2. In particular, the invention provides antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprising, or alternatively consisting of, a polypeptide having the amino acid sequence of a VH CDR1 contained in a heavy chain expressed by one or more cell lines referred to in Table 2. In another embodiment, antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a VH CDR2 contained in a heavy chain expressed by one or more cell lines referred to in Table 2. In a

preferred embodiment, antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprise, or alternatively consist of a polypeptide having the amino acid sequence of a VH CDR3 contained in a heavy chain expressed by one or more cell lines referred to in Table 2. Molecules comprising, or alternatively consisting of, these antibodies, or antibody fragments or variants thereof, that immunospecifically bind to G-protein Chemokine Receptor (CCR5) or a G-protein Chemokine Receptor (CCR5) fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments and/or variants.

The present invention also provides antibodies that immunospecifically bind to a polypeptide, or polypeptide fragment or variant of a G-protein Chemokine Receptor (CCR5), wherein said antibodies comprise, or alternatively consist of, a polypeptide having an amino acid sequence of any one, two, three, or more of the VL CDRs contained in a light chain expressed by one or more cell lines referred to in Table 2. In particular, the invention provides antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprising, or alternatively consisting of, a polypeptide having the amino acid sequence of a VL CDR1 contained in a light chain expressed by one or more cell lines referred to in Table 2. In another embodiment, antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprise, or alternatively consist of, a polypeptide having the amino acid sequence of a VL CDR2 contained in a light chain expressed by one or more cell lines referred to in Table 2. In a preferred embodiment, antibodies that immunospecifically bind a G-protein Chemokine Receptor (CCR5), comprise, or alternatively consist of a polypeptide having the amino acid sequence of a VL CDR3 contained in a light chain expressed by one or more cell lines referred to in Table 2. Molecules comprising, or alternatively consisting of, these antibodies, or antibody fragments or variants thereof, that immunospecifically bind to G-protein Chemokine Receptor (CCR5) or a G-protein Chemokine Receptor (CCR5) fragment or variant thereof are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments and/or variants.

The present invention also provides antibodies (including molecules comprising, or alternatively consisting of, antibody fragments or variants) that immunospecifically bind to a G-protein Chemokine Receptor (CCR5) polypeptide or polypeptide fragment or variant of a G-protein Chemokine Receptor (CCR5), wherein said antibodies comprise, or alternatively consist of, one, two, three, or more VH CDRs and one, two, three or more VL CDRs, as contained in a heavy chain or light chain expressed by one or more cell lines referred to in Table 2. In particular, the invention provides for antibodies that immunospecifically bind to a polypeptide or polypeptide fragment or variant of a G-protein Chemokine Receptor (CCR5), wherein said antibodies comprise, or alternatively consist of, a VH CDR1 and a VL CDR1, a VH CDR1 and a VL CDR2, a VH CDR1 and a VL CDR3, a VH CDR2 and a VL CDR1, VH CDR2 and VL CDR2, a VH CDR2 and a VL CDR3, a VH CDR3 and a VH CDR1, a VH CDR3 and a VL CDR2, a VH CDR3 and a VL CDR3, or any combination thereof, of the VH CDRs and VL CDRs contained in a heavy chain or light chain expressed by one or more cell lines referred to in Table 2. In a preferred embodiment, one or more of these combinations are from the same scFv as disclosed in Table 2. Molecules comprising, or alternatively consisting of, fragments or variants of these antibodies, that immunospecifically bind to G-protein Chemokine Receptor (CCR5) are also encompassed by the invention, as are nucleic acid molecules encoding these antibodies, molecules, fragments or variants.

Nucleic Acid Molecules Encoding anti-G-protein Chemokine Receptor (CCR5) Antibodies Corresponding to Antibodies Derived from Xenomouse Strains.

The present invention also provides for nucleic acid molecules, generally isolated, encoding an antibody of the invention (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof). In a specific embodiment, a nucleic acid molecule of the invention encodes an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), comprising, or alternatively consisting of, a VH domain having an amino acid sequence of any one of the VH domains of a heavy chain expressed by at least one of the cell lines referred to in Table 2 and a VL domain having an amino acid sequence of a light chain expressed by at

least one of the cell lines referred to in Table 2. In another embodiment, a nucleic acid molecule of the invention encodes an antibody (including molecules comprising, or alternatively consisting of, antibody fragments or variants thereof), comprising, or alternatively consisting of, a VH domain having an amino acid sequence of any one of the VH domains of a heavy chain expressed by at least one of the cell lines referred to in Table 2 or a VL domain having an amino acid sequence of a light chain expressed by at least one of the cell lines referred to in Table 2.

The present invention also provides antibodies that comprise, or alternatively consist of, variants (including derivatives) of the antibody molecules (e.g., the VH domains and/or VL domains) described herein, which antibodies immunospecifically bind to a G-protein Chemokine Receptor (CCR5) or fragment or variant thereof. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH domain, VHCDR1, VHCDR2, VHCDR3, VL domain, VLCDR1, VLCDR2, or VLCDR3. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains

(e.g., tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (e.g., the ability to bind a G-protein Chemokine Receptor).

For example, it is possible to introduce mutations only in framework regions or only in CDR regions of an antibody molecule. Introduced mutations may be silent or neutral missense mutations, i.e., have no, or little, effect on an antibody's ability to bind antigen. These types of mutations may be useful to optimize codon usage, or improve a hybridoma's antibody production. Alternatively, non-neutral missense mutations may alter an antibody's ability to bind antigen. The location of most silent and neutral missense mutations is likely to be in the framework regions, while the location of most non-neutral missense mutations is likely to be in CDR, though this is not an absolute requirement. One of skill in the art would be able to design and test mutant molecules with desired properties such as no alteration in antigen binding activity or alteration in binding activity (e.g., improvements in antigen binding activity or change in antibody specificity). Following mutagenesis, the encoded protein may routinely be expressed and the functional and/or biological activity of the encoded protein, (e.g., ability to immunospecifically bind a G-protein Chemokine Receptor) can be determined using techniques described herein or by routinely modifying techniques known in the art.

In a specific embodiment, an antibody of the invention (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds G-protein Chemokine Receptor (CCR5) polypeptides or fragments or variants thereof, comprises, or alternatively consists of, an amino acid sequence encoded by a nucleotide sequence that hybridizes to a nucleotide sequence that is complementary to that encoding one of the VH or VL domains expressed by one or more cell lines referred to in Table 2. under stringent conditions, e.g., hybridization to filter-bound DNA in 6X sodium chloride/sodium citrate (SSC) at about 45° C followed by one or more washes in 0.2xSSC/0.1% SDS at about 50-65° C, under highly stringent conditions, e.g., hybridization to filter-bound nucleic acid in 6xSSC at about 45° C

followed by one or more washes in 0.1xSSC/0.2% SDS at about 68° C, or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds. , 1989, *Current Protocols in Molecular Biology*, Vol. I, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., New York at pages 6.3.1-6.3.6 and 2.10.3). Nucleic acid molecules encoding these antibodies are also encompassed by the invention.

It is well known within the art that polypeptides, or fragments or variants thereof, with similar amino acid sequences often have similar structure and many of the same biological activities. Thus, in one embodiment, an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds to a G-protein Chemokine Receptor (CCR5) polypeptide or fragments or variants of a G-protein Chemokine Receptor (CCR5) polypeptide, comprises, or alternatively consists of, a VH domain having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to the amino acid sequence of a VH domain of a heavy chain expressed by at least one of the cell lines referred to in Table 2.

In another embodiment, an antibody (including a molecule comprising, or alternatively consisting of, an antibody fragment or variant thereof), that immunospecifically binds to a G-protein Chemokine Receptor (CCR5) polypeptide or fragments or variants of a G-protein Chemokine Receptor (CCR5) polypeptide, comprises, or alternatively consists of, a VL domain having an amino acid sequence that is at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical, to the amino acid sequence of a VL domain of a light chain expressed by at least one of the cell lines referred to in Table 2.

Polynucleotides Encoding Antibodies

Antibodies of the invention (including antibody fragments or variants) can be produced by any method known in the art. For example, it will be appreciated that antibodies in accordance with the present invention can be expressed in cell lines other than hybridoma cell lines, including but not limited to myeloma cell lines. Sequences encoding the cDNAs or genomic clones for the particular antibodies can be used for transformation of a suitable mammalian or nonmammalian host cells or to generate phage display libraries, for example. Additionally, polypeptide antibodies of the invention may be chemically synthesized or produced through the use of recombinant expression systems.

One way to produce the antibodies of the invention would be to clone the VH and/or VL domains expressed by any one or more of the hybridoma cell lines referred to in Table 2. In order to isolate the VH and VL domains from the hybridoma cell lines, PCR primers including VH or VL nucleotide sequences (See Example 55), may be used to amplify the expressed VH and VL sequences contained in total RNA isolated from hybridoma cell lines. The PCR products may then be cloned using vectors, for example, which have a PCR product cloning site consisting of a 5' and 3' single T nucleotide overhang, that is complementary to the overhanging single adenine nucleotide added onto the 5' and 3' end of PCR products by many DNA polymerases used for PCR reactions. The VH and VL domains can then be sequenced using conventional methods known in the art.

The cloned VH and VL genes may be placed into one or more suitable expression vectors. By way of non-limiting example, PCR primers including VH or VL nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site may be used to amplify the VH or VL sequences. Utilizing cloning techniques known to those of skill in the art, the PCR amplified VH domains may be cloned into vectors expressing the appropriate immunoglobulin constant region, *e.g.*, the human IgG1 or IgG4 constant region for VH domains, and the human kappa or lambda constant regions for kappa and lambda VL domains, respectively. Preferably, the vectors for expressing the VH or VL

domains comprise a promoter suitable to direct expression of the heavy and light chains in the chosen expression system, a secretion signal, a cloning site for the immunoglobulin variable domain, immunoglobulin constant domains, and a selection marker such as neomycin. The VH and VL domains may also be cloned into a single vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, *e.g.*, IgG, using techniques known to those of skill in the art (See, for example, Guo et al., J. Clin. Endocrinol. Metab. 82:925-31 (1997), and Ames et al., J. Immunol. Methods 184:177-86 (1995) which are herein incorporated in their entireties by reference).

Polynucleotides encoding any of the VH or VL domains of the invention can be combined with sequences known in the art to generate an expression vector encoding an antibody molecule or portion thereof. For example, a polynucleotide sequence encoding a VH domain can be combined with a polynucleotide sequence encoding an immunoglobulin constant domain, such as a human IgG1 heavy chain constant domain or a human IgG4 heavy chain constant domain. A polynucleotide sequence encoding a VL domain can be combined with a polynucleotide sequence encoding an immunoglobulin constant domain, such as a human kappa light chain constant domain or a human Lambda light chain constant domain. The amino acid sequences of human IgG1 heavy chain constant domain (SEQ ID NO:81), human IgG4 heavy chain constant domain (SEQ ID NO:82), and human kappa light chain constant domain (SEQ ID NO:83) are shown below. The expression vector for an antibody molecule can also encode a signal sequence at the N-terminal end of the sequences encoding the VH and/or VL domains to promote secretion into the culture medium. Examples of such signal sequences are provided below (SEQ ID NOS:84-85).

Human IgG1 Heavy Chain Constant Domains (SEQ ID NO:81)

ASTKGPSVFP	LAPSSKSTSG	GTAALGCLVK	DYFPEPVTVS	WNSGALTSGV	HTFPAVLQSS	60
GLYSLSSVVT	VPSSSLGTQT	YICNVNHKPS	NTKVDKKVEP	KSCDKTHTCP	PCPAPELLGG	120

PSVFLEPPKP	KDTLMIS RTP	EVTCVVVDVS	HEDPEVKFNW	YVDGVEVHNA	KTKPREEQYN	180
STYRVVSVLT	VLHQDWLNGK	EYCKVSNKA	LPAPIEKTIS	KAKGQPREPQ	VYTLPPSRDE	240
LTKNQVSLTC	LVKGFYPSDI	AVEWESNGQP	ENNYKTTPPV	LDSDGSFFLY	SKLTVDKSRW	300
QQGNVFSCSV	MHEALHNHYT	QKSLSLSPGK				330

Human IgG4 Heavy Chain Constant Domains (SEQ ID NO:82)

ASTKGPSVFP	LAPCSRSTSE	STAALGCLVK	DYFPEPVTVS	WNSGALTSGV	HTFPAVLQSS	60
GLYSLSSVAT	VPSSSLGTKT	YTCNVDPKPS	NTKVDKRVES	KYGPPCPSCP	APEFLGGPSV	120
FLFPPKPKDT	LMISRTPEVT	CVVVDVSQED	PEVQFNWYVD	GVEVHNAKTK	PREEQFNSTY	180
RVVSVLTVLH	QDWLNGKEYK	CKVSNKGLPS	SIEKTISKAK	GQPREPQVYT	LPPSQEEMTK	240
NQVSLTCLVK	GFYPSDIAVE	WESNGQPENN	YKTTTPVLDS	DGSFFLYSRL	TVDKSRWQEG	300
NVFSCSVMHE	ALHNHYTQKS	LSLSLGK				327

Human Kappa Constant Domain (SEQ ID NO:83)

TVAAPSVFIF	PPSDEQLKSG	TASVVCLLNN	FYPREAKVQW	KVDNALQSGN	SQESVTEQDS	60
KDSTYLSST	LTLKADYEK	HKVYACEVTH	QGLSSPVTKS	FNRGEC		106

Signal Peptides for Immunoglobulin Heavy or Light Chains

MGWSCIIIFL	VATATGAHS	19
(SEQ ID NO:84)		

MGWSCIIIFL	VATATGVHS	19
(SEQ ID NO:85)		

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:2 or a polypeptide encoded by the deposited clone.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A⁺ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence (See Example 55) or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well known in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., *J. Mol. Biol.*

278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

Exemplary alterations which can be introduced into the human IgG4 constant region include (amino acid positions correspond to SEQ ID NO:82): A69 replaced with V, L76 replaced with F, K79 replaced with Q, R97 replaced with T, S100 replaced with R, Y102 replaced with C, G103 replaced with C, P104 replaced with V, P105 replaced with E, S108 replaced with P, E113 replaced with P, L115 replaced with E or V, G116 replaced with A, Q148 replaced with H, V162 replaced with M, Y180 replaced with F, L189 replaced with V, S210 replaced with A, S211 replaced with P, A219 replaced with T, Q235 replaced with R, A258 replaced with S, V277 replaced with M, R289 replaced with K, E299 replaced with Q, V302 replaced with I, and/or L325 replaced with P. Reddy et al. have reported that the substitution of L115 with E and of S108 with P reduce Fc binding and CD4+ T cell depletion (M.P. Reddy et al., J Immunol 164:1925-1933 (2000)).

For some uses, such as for in vitro affinity maturation of an antibody of the invention, it may be useful to express the VH and VL domains of the heavy and light chains of one or more antibodies of the invention as single chain antibodies or Fab fragments in a phage display library. For example, the cDNAs encoding the VH and VL domains of one or more antibodies of the invention may be expressed in all possible combinations using a phage display library, allowing for the selection of VH/VL combinations that bind a G-protein Chemokine Receptor (CCR5) polypeptides with preferred binding characteristics such as improved affinity or improved off rates. Additionally, VH and VL segments - the CDR regions of the VH and VL domains of one or more antibodies of the invention, in particular, may be mutated in vitro. Expression of

VH and VL domains with "mutant" CDRs in a phage display library allows for the selection of VH/VL combinations that bind a G-protein Chemokine Receptor (CCR5) receptor polypeptides with preferred binding characteristics such as improved affinity or improved off rates.

In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding VH and VL domains are amplified from animal cDNA libraries (*e.g.*, human or murine cDNA libraries of lymphoid tissues) or synthetic cDNA libraries. The DNA encoding the VH and VL domains are joined together by an scFv linker by PCR and cloned into a phagemid vector (*e.g.*, p CANTAB 6 or pComb 3 HSS). The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the VH and VL domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to an antigen of interest (*i.e.*, a G-protein Chemokine Receptor polypeptide or a fragment thereof) can be selected or identified with antigen, *e.g.*, using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies of the present invention include, but are not limited to, those disclosed in Brinkman *et al.*, J. Immunol. Methods 182:41-50 (1995); Ames *et al.*, J. Immunol. Methods 184:177-186 (1995); Kettleborough *et al.*, Eur. J. Immunol. 24:952-958 (1994); Persic *et al.*, Gene 187 9-18 (1997); Burton *et al.*, Advances in Immunology 57:191-280(1994); PCT application No. PCT/GB91/O1 134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18719; WO 93/1 1236; WO 95/15982; WO 95/20401; WO97/13844; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,717; 5,780,225; 5,658,727; 5,735,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison *et al.*, Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger *et al.*, Nature 312:604-608 (1984); Takeda *et al.*, Nature 314:452-454 (1985)) by splicing 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. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423-42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. 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. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., Science 242:1038-1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis, by intracellular immunization (i.e., intrabody technology), or preferably, by recombinant expression techniques. Methods of producing antibodies include, but are not limited to, hybridoma technology, EBV transformation, and other methods discussed herein as well as through the use recombinant DNA technology, as discussed below.

Recombinant expression of an antibody of the invention, or fragment, derivative, variant or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods

include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3, NSO cells) harboring recombinant expression constructs

containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, *Proc. Natl. Acad. Sci. USA* 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., *Methods in Enzymol.* 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be

engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp^rt- or ap^rt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215; and hyg^r, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human

Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., *J. Mol. Biol.* 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., *Mol. Cell. Biol.* 3:257 (1983)).

Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulphoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. Vectors that use glutamine synthase as the selectable marker include, but are not limited to, the pEE6 expression vector described in Stephens and Cockett, *Nucl. Acids. Res* 17:7110 (1989). A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suppliers, including, for example Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are incorporated in their entireties by reference herein.

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical

selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature* 322:52 (1986); Kohler, *Proc. Natl. Acad. Sci. USA* 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

Antibodies of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the antibodies of the present invention may be glycosylated or may be non-glycosylated. In addition, antibodies of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Antibodies of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller, M., et al., 1984, *Nature* 310:105-111). For example, a peptide corresponding to a fragment of an antibody of the invention can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the antibody polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino

hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses antibodies which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄, acetylation, formylation, oxidation, reduction, metabolic synthesis in the presence of tunicamycin, etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The antibodies may also be modified with a detectable label, such as an enzymatic, fluorescent, radioisotopic or affinity label to allow for detection and isolation of the antibody.

Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, glucose oxidase or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include biotin, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include a radioactive metal ion, e.g., alpha-emitters such as, for example, ²¹³Bi, or other radioisotopes such as, for example, iodine (¹³¹I, ¹²⁵I, ¹²³I, ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (^{115m}In, ^{113m}In, ¹¹²In, ¹¹¹In), and technetium

(⁹⁹Tc, ^{99m}Tc), thallium (²⁰¹Tl), gallium (⁶⁸Ga, ⁶⁷Ga), palladium (¹⁰³Pd), molybdenum (⁹⁹Mo), xenon (¹³³Xe), fluorine (¹⁸F), ¹⁵³Sm, ¹⁷⁷Lu, ¹⁵⁹Gd, ¹⁴⁹Pm, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁶⁶Ho, ⁹⁰Y, ⁴⁷Sc, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁴²Pr, ¹⁰⁵Rh, ⁹⁷Ru, ⁶⁸Ge, ⁵⁷Co, ⁶⁵Zn, ⁸⁵Sr, ³²P, ¹⁵³Gd, ¹⁶⁹Yb, ⁵¹Cr, ⁵⁴Mn, ⁷⁵Se, ¹¹³Sn, and ¹¹⁷Tm.

In specific embodiments, antibodies of the invention may be labeled with Europium. For example, antibodies of the invention may be labelled with Europium using the DELFIA Eu-labeling kit (catalog# 1244-302, Perkin Elmer Life Sciences, Boston, MA) following manufacturer's instructions.

In specific embodiments, antibodies of the invention are attached to macrocyclic chelators useful for conjugating radiometal ions, including but not limited to, ¹¹¹In, ¹⁷⁷Lu, ⁹⁰Y, ¹⁶⁶Ho, ¹⁵³Sm, ²¹⁵Bi and ²²⁵Ac to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators attached to antibodies of the invention is ¹¹¹In. In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator attached to antibodies polypeptides of the invention is ⁹⁰Y. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In specific embodiments, the macrocyclic chelator is α -(5-isothiocyanato-2-methoxyphenyl)-1,4,7,10-tetraaza-cyclododecane-1,4,7,10-tetraacetic acid. In other specific embodiments, the DOTA is attached to the antibody of the invention via a linker molecule. Examples of linker molecules useful for conjugating a macrocyclic chelator such as DOTA to a polypeptide are commonly known in the art -- see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90, 1998; Peterson et al., Bioconjug. Chem. 10(4):553-7, 1999; and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50, 1999 which are hereby incorporated by reference in their entirety. In addition, U.S. Patents 5,652,361 and 5,756,065, which disclose chelating agents that may be conjugated to antibodies, and methods for making and using them, are hereby incorporated by reference in their entirety.

In one embodiment, antibodies of the invention are labeled with biotin. In other related embodiments, biotinylated antibodies of the invention may be used, for example, as an imaging agent or as a means of identifying one or more TRAIL receptor coreceptor or ligand molecules.

Also provided by the invention are chemically modified derivatives of antibodies of the invention which may provide additional advantages such as increased solubility, stability and in vivo or in vitro circulating time of the polypeptide, or decreased immunogenicity (see U. S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The antibodies may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo et al., *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev et al., *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti et al., *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the antibody with consideration of effects on functional or antigenic domains of the antibody. There are a number of attachment methods available to those skilled in the art,

e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include, for example, lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins, e.g., antibodies, via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire antibodies chemically modified at the N-terminus of either the heavy chain or the light chain or both. Using polyethylene glycol as an illustration, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective chemical modification at the N-terminus may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate

reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the antibodies of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the antibody either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., *Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992); Francis et al., *Intern. J. of Hematol.* 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of antibodies without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($\text{ClSO}_2\text{CH}_2\text{CF}_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes antibody-polyethylene glycol conjugates produced by reacting antibodies of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to antibodies using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Antibody-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the antibody by a linker can also be produced by reaction of antibodies with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated antibody products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each antibody of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated antibodies of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15,

17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per antibody molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992).

Antibody Conjugates

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide). Antibodies of the invention may also be fused to albumin (including but not limited to recombinant human serum albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an

integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention. Such fusion proteins may, for example, facilitate purification and may increase half-life in vivo. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095; Naramura et al., *Immunol. Lett.* 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., *PNAS* 89:1428-1432 (1992); Fell et al., *J. Immunol.* 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., *Proc. Natl. Acad. Sci. USA* 88:10535-10539 (1991); Zheng et al., *J. Immunol.* 154:5590-5600 (1995); and Vil et al., *Proc. Natl. Acad. Sci. USA* 89:11337-11341(1992) (said references incorporated by reference in their entireties).

As discussed, *supra*, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:2 or of the polypeptide encoded by the deposited clone may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO: 2 or to the polypeptide encoded

by the deposited clone may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., *Nature* 331:84-86 (1988)). The polypeptides of the present invention fused or conjugated to an antibody having disulfide-linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995)).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., *Proc. Natl. Acad. Sci. USA* 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., *Cell* 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection

can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include iodine (^{121}I , ^{123}I , ^{125}I , ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{111}In , ^{112}In , $^{113\text{m}}\text{In}$, $^{115\text{m}}\text{In}$), technetium (^{99}Tc , $^{99\text{m}}\text{Tc}$), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , and ^{97}Ru ;

In specific embodiments, G-protein Chemokine Receptor (CCR5) polypeptides of the invention are attached to macrocyclic chelators useful for conjugating radiometal ions, including but not limited to, ^{111}In , ^{177}Lu , ^{90}Y , ^{166}Ho , and ^{153}Sm , to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators attached to G-protein Chemokine Receptor (CCR5) polypeptides of the invention is ^{111}In . In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator attached to G-protein Chemokine Receptor (CCR5) polypeptides of the invention is ^{90}Y . In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, the DOTA is attached to the G-protein Chemokine Receptor (CCR5) polypeptide of the invention via a linker molecule. Examples of linker molecules useful

for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90, 1998; Peterson et al., Bioconjug. Chem. 10(4):553-7, 1999; and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50, 1999 which are hereby incorporated by reference in their entirety. In addition, U.S. Patents 5,652,361 and 5,756,065, which disclose chelating agents that may be conjugated to antibodies, and methods for making and using them, are hereby incorporated by reference in their entireties. Though U.S. Patents 5,652,361 and 5,756,065 focus on conjugating chelating agents to antibodies, one skilled in the art could readily adapt the methods disclosed therein in order to conjugate chelating agents to other polypeptides.

A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The antibody conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF- α , TNF- β , AIM I (See, International Publication No. WO 97/33899), AIM II (See,

International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as BIAcore analysis (see, e.g., Example 59), FACS (Fluorescence activated cell sorter) analysis (see, e.g., Example 54), immunofluorescence (see, e.g., Example 56), immunocytochemistry, western blots (see Examples 64 and 65), radioimmunoassays, ELISA (enzyme linked immunosorbent assay) (See, e.g., Example 54), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds,

1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasyolol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%-20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., ³²P or ¹²⁵I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g.,

Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., ^3H or ^{125}I), or fragment or variant thereof, with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a G-protein Chemokine Receptor (CCR5) and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the G-protein Chemokine Receptor (CCR5) is incubated with antibody of interest conjugated to a labeled compound (e.g., compound labeled with ^3H or ^{125}I) in the presence of increasing amounts of an unlabeled second antibody. This kind of competitive assay between two antibodies, may also be used to determine if two antibodies bind the same, closely associated (e.g., overlapping) or different epitopes.

In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of antibodies (including antibody fragments or variants thereof) to

a G-protein Chemokine Receptor (CCR5), or fragments of a G-protein Chemokine Receptor (CCR5). BIAcore kinetic analysis comprises analyzing the binding and dissociation of antibodies from chips with immobilized G-protein Chemokine Receptors (CCR5) on their surface as described in Example 59.

Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic

growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy, anti-tumor agents, and anti-retroviral agents (see Example 28, below). In a highly preferred embodiment, antibodies of the invention may be administered alone or in combination with and anti-retroviral agents (see Example 28, below). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M. More preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M or 10^{-8} M. Even more preferred binding affinities include those with a dissociation constant or K_d less than 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this

embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., *Clinical Pharmacy* 12:488-505 (1993); Wu and Wu, *Biotherapy* 3:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596 (1993); Mulligan, *Science* 260:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem.* 62:191-217 (1993); May, *TIBTECH* 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); Zijlstra et al., *Nature* 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid-carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the *mdr1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy

are: Clowes et al., *J. Clin. Invest.* 93:644-651 (1994); Kiem et al., *Blood* 83:1467-1473 (1994); Salmons and Gunzberg, *Human Gene Therapy* 4:129-141 (1993); and Grossman and Wilson, *Curr. Opin. in Genetics and Devel.* 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, *Current Opinion in Genetics and Development* 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., *Human Gene Therapy* 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., *Science* 252:431-434 (1991); Rosenfeld et al., *Cell* 68:143-155 (1992); Mastrangeli et al., *J. Clin. Invest.* 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., *Gene Therapy* 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., *Proc. Soc. Exp. Biol. Med.* 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the

art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, *Meth. Enzymol.* 217:599-618 (1993); Cohen et al., *Meth. Enzymol.* 217:618-644 (1993); Cline, *Pharmac. Ther.* 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, *Cell* 71:973-985 (1992); Rheinwald, *Meth. Cell Bio.* 21A:229 (1980); and Pittelkow and Scott, *Mayo Clinic Proc.* 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. **Demonstration of Therapeutic or Prophylactic Activity**

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., *Macromol. Sci. Rev. Macromol. Chem.* 23:61 (1983); see also Levy et al., *Science* 228:190 (1985); During et al., *Ann. Neurol.* 25:351 (1989); Howard et al., *J. Neurosurg.* 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., *Proc. Natl. Acad. Sci. USA* 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized

pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it

can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of

pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay

(ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{125}I , ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{112}In), and technetium (^{99}Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of $^{99\text{m}}\text{Tc}$. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled

molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may

be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum

components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Fusion Proteins

Any G-protein Chemokine Receptor (CCR5) polypeptide can be used to generate fusion proteins. For example, the G-protein Chemokine Receptor (CCR5) polypeptide, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the G-protein Chemokine Receptor (CCR5) polypeptide can be used to indirectly detect the second protein by binding to the G-protein Chemokine Receptor. Moreover, because secreted proteins target cellular locations based on trafficking signals, the G-protein Chemokine Receptor (CCR5) polypeptides can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to G-protein Chemokine Receptor (CCR5) polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

In certain preferred embodiments, G-protein Chemokine Receptor (CCR5) proteins of the invention comprise fusion proteins wherein the G-protein Chemokine Receptor (CCR5) polypeptides are those described above as m-n. In preferred embodiments, the application is directed to nucleic acid molecules at least 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides having the amino acid sequence of the specific N- and C-terminal deletions recited herein. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Moreover, fusion proteins may also be engineered to improve characteristics of the G-protein Chemokine Receptor (CCR5) polypeptide. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the G-protein Chemokine Receptor (CCR5) polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the G-protein Chemokine Receptor (CCR5) polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the G-protein Chemokine Receptor (CCR5) polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

As one of skill in the art will appreciate, polypeptides of the present invention and the epitope-bearing fragments thereof described above, can be combined with heterologous polypeptide sequences. . For example, polypeptides of the present invention (including fragments or variants thereof), may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof, resulting in chimeric polypeptides. By way of another non-limiting example, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused with albumin (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 – 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another

preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide). Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention.

These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827; Traunecker et al., *Nature* 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., *J. Biochem.* 270:3958-3964 (1995).)

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., *J. Molecular Recognition* 8:52-58 (1995); K. Johanson et al., *J. Biol. Chem.* 270:9459-9471 (1995).)

Moreover, the G-protein Chemokine Receptor (CCR5) polypeptides can be fused to marker sequences, such as a peptide which facilitates purification of G-protein Chemokine Receptor. In preferred embodiments, the marker amino acid sequence is a

hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the G-protein Chemokine Receptor (CCR5) polynucleotides or the polypeptides.

Vectors, Host Cells, and Protein Production

The present invention also relates to vectors containing the G-protein Chemokine Receptor (CCR5) polynucleotide, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

G-protein Chemokine Receptor (CCR5) polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The G-protein Chemokine Receptor (CCR5) polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418, glutamine synthase or

neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g., *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, NSO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulfoximine or methotrexate, respectively. The availability of drugs which inhibit the function of the enzymes encoded by these selectable markers allows for selection of cell lines in which the vector sequences have been amplified after integration into the host cell's DNA. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. Vectors that use glutamine synthase as the selectable marker include the pEE6 expression vector described in Stephens and Cockett, *Nucl. Acids. Res* 17:7110 (1989). A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are hereby incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suppliers including, for example, Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are herein incorporated by reference.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a,

pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that G-protein Chemokine Receptor (CCR5) polypeptides may in fact be expressed by a host cell lacking a recombinant vector.

G-protein Chemokine Receptor (CCR5) polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

G-protein Chemokine Receptor (CCR5) polypeptides, and preferably the secreted form, can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the G-protein Chemokine Receptor (CCR5) polypeptides may be glycosylated or may be non-glycosylated. In addition, G-protein Chemokine Receptor (CCR5) polypeptides may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-

terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express G-protein Chemokine Receptor (CCR5) protein in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O₂. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O₂. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See, Ellis, S.B., *et al.*, *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., *et al.*, *Yeast* 5:167-77 (1989); Tschopp, J.F., *et al.*, *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a G-protein Chemokine Receptor (CCR5) polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a G-protein Chemokine Receptor (CCR5) polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a G-protein Chemokine Receptor (CCR5) protein of the invention by virtue of the strong *AOX1* promoter linked to the

Pichia pastoris alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a G-protein Chemokine Receptor (CCR5) polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., G-protein Chemokine Receptor (CCR5) coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with G-protein Chemokine Receptor (CCR5) polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous G-protein Chemokine Receptor (CCR5) polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous G-protein Chemokine Receptor (CCR5) polynucleotide sequences via homologous recombination, resulting in the formation of a new transcription unit (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; U.S. Patent No. 5,733,761, issued March 31, 1998; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a G-protein Chemokine Receptor (CCR5) polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the G-protein Chemokine Receptor (CCR5) polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, γ -Abu, ϵ -Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses G-protein Chemokine Receptor (CCR5) polypeptides which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH_4 ; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog).

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., *Exp. Hematol.* 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

The G-protein Chemokine Receptor (CCR5) polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the G-protein Chemokine Receptor (CCR5) polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:2 or encoded by the HDG NR10 DNA contained in the deposited clone (including fragments, variants, splice variants, and fusion proteins, corresponding to these as described herein). These homomers may contain G-protein Chemokine Receptor (CCR5) polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only G-protein Chemokine Receptor (CCR5) polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the

invention is a multimer containing G-protein Chemokine Receptor (CCR5) polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing G-protein Chemokine Receptor (CCR5) polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing G-protein Chemokine Receptor (CCR5) polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the G-protein Chemokine Receptor (CCR5) polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the G-protein Chemokine Receptor (CCR5) polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:2, or contained in the polypeptide encoded by the clone HDGNR10). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a G-protein Chemokine Receptor (CCR5) fusion

protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a G-protein Chemokine Receptor-Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference.

Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a

linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Uses of the G-protein Chemokine Receptor (CCR5) Polynucleotides

The G-protein Chemokine Receptor (CCR5) polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the sequences shown in SEQ ID NO:1 or from the deposited clone. Primers can be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human G-protein Chemokine Receptor (CCR5) gene corresponding to the SEQ ID NO:1 or to the deposited clone will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the G-protein Chemokine Receptor (CCR5) polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, and preselection by hybridization to construct chromosome specific-cDNA libraries.

Precise chromosomal location of the G-protein Chemokine Receptor (CCR5) polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the G-protein Chemokine Receptor (CCR5) polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes). Preferred polynucleotides correspond to the noncoding regions of the cDNAs or genomic clone because the coding sequences are more likely conserved within gene families, thus increasing the chance of cross hybridization during chromosomal mapping.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library) .) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in the G-protein Chemokine Receptor (CCR5) polynucleotide and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the G-protein Chemokine Receptor (CCR5) polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using G-protein Chemokine Receptor (CCR5) polynucleotides. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the present invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the present invention, where each probe has one strand containing a 31' mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a disorder, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the present invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotide of the present invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the present invention or the level of the mRNA encoding the polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in

the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having a disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains the polypeptide of the present invention or mRNA. As indicated, biological samples include body fluids (such as semen, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and other tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the present invention attached may be used to identify polymorphisms between the polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, including cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available

commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, *Science* 254, 1497 (1991); and M. Egholm, O. Buchardt, L. Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden, and P. E. Nielsen, *Nature* 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (T_m) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention is useful for detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Germann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in *Neoplastic Diseases of the Blood*, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by

chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelman et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelman et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelman et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580) However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness would not be limited to treatment of proliferative diseases, disorders, and/or conditions of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a G-protein Chemokine Receptor (CCR5) polynucleotide can be used to control gene expression through triple helix formation or antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem.

56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat or prevent disease.

G-protein Chemokine Receptor (CCR5) polynucleotides are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. G-protein Chemokine Receptor (CCR5) offers a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The G-protein Chemokine Receptor (CCR5) polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The G-protein Chemokine Receptor (CCR5) polynucleotides can be used as additional DNA markers for RFLP.

The G-protein Chemokine Receptor (CCR5) polynucleotides can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once a unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as

tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, G-protein Chemokine Receptor (CCR5) polynucleotides can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to particular tissue prepared from G-protein Chemokine Receptor (CCR5) sequences. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

Because G-protein Chemokine Receptor (CCR5) is expressed in macrophages and memory T cells, G-protein Chemokine Receptor (CCR5) polynucleotides are useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to G-protein Chemokine Receptor (CCR5) polypeptides are useful to provide immunological probes for differential identification of the tissue(s) or cell type(s). In addition, for a number of diseases, disorders, and/or conditions of the above tissues or cells, or in which these cells play a role, significantly higher or lower levels of G-protein Chemokine Receptor (CCR5) gene expression may be detected in certain tissues (e.g., cancerous and wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" G-protein Chemokine Receptor (CCR5) gene expression level, i.e., the G-protein Chemokine Receptor (CCR5) expression level in healthy tissue from an individual not having the immune system-related disorder.

Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying G-protein Chemokine Receptor (CCR5) gene expression level in cells or body fluid of an individual; (b) comparing the G-protein Chemokine Receptor (CCR5) gene

expression level with a standard G-protein Chemokine Receptor (CCR5) gene expression level, whereby an increase or decrease in the assayed G-protein Chemokine Receptor (CCR5) gene expression level compared to the standard expression level is indicative of disorder in the immune system or related to the immune system.

In a further embodiment, the invention provides a method of using G-protein Chemokine Receptor (CCR5) polynucleotides, or fragments or variants thereof as a vaccine to elicit an immune response to G-protein Chemokine Receptor. In a preferred embodiment, the invention provides a method of using G-protein Chemokine Receptor (CCR5) polynucleotides, or fragments or variants thereof as a DNA vaccine to elicit a humoral (antibody-mediated) immune response to G-protein Chemokine Receptor. In other highly preferred embodiments, the invention provides a method of using G-protein Chemokine Receptor (CCR5) polynucleotides comprising the nucleotide sequence of one or more extracellular loops of G-protein Chemokine Receptor (CCR5) (i.e., amino acids 89-102, 167-195 and/or 261-274 of SEQ ID NO:2 or of the polypeptide encoded by the deposited clone (SEQ ID NO:22)) as a DNA vaccine to elicit immune response to G-protein Chemokine Receptor. In other highly preferred embodiments, the invention provides a method of using G-protein Chemokine Receptor (CCR5) polynucleotides comprising the nucleotide sequence of the first extracellular loop of G-protein Chemokine Receptor (CCR5) (i.e., amino acids 89-102 of SEQ ID NO:2 or of the polypeptide encoded by the deposited clone (SEQ ID NO:22)) as a DNA vaccine to elicit an immune response to G-protein Chemokine Receptor (CCR5). In other highly preferred embodiments, the invention provides a method of using G-protein Chemokine Receptor (CCR5) polynucleotides comprising the nucleotide sequence of the second extracellular loop of G-protein Chemokine Receptor (CCR5) (i.e., amino acids 167-195 of SEQ ID NO:2 or of the polypeptide encoded by the deposited clone (SEQ ID NO:22)) as a DNA vaccine to elicit an immune response to G-protein Chemokine Receptor (CCR5). In other highly preferred embodiments, the invention provides a method of using G-protein Chemokine Receptor (CCR5) polynucleotides comprising the nucleotide sequence of the third extracellular loop of G-protein Chemokine Receptor (CCR5) (i.e., 261-274 of SEQ ID NO:2 or of the polypeptide encoded by the deposited clone (SEQ ID NO:22)) as a DNA vaccine to elicit an immune response to G-protein Chemokine Receptor (CCR5).

DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

CECI EST LE TOME __1__ DE __3__

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JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __3__

NOTE: For additional volumes please contact the Canadian Patent Office.

What Is Claimed Is:

1. An antibody that immunospecifically binds G-protein Chemokine Receptor (CCR5) polypeptide consisting of a first amino acid sequence and a second amino acid sequence, said first amino acid sequence comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to an amino acid sequence of the VH domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5861 and said second amino acid sequence comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to an amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5861.

2. An antibody that immunospecifically binds G-protein Chemokine Receptor (CCR5) polypeptide consisting of a first amino acid sequence and a second amino acid sequence, said first amino acid sequence comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:76 and said second amino acid sequence comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to an amino acid sequence of SEQ ID NO:80.

3. An antibody that immunospecifically binds G-protein Chemokine Receptor (CCR5) polypeptide, said antibody comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of the VH domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5861;
- (b) the amino acid sequence of the VH domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5860;
- (c) the amino acid sequence of the VH domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5859;

(d) the amino acid sequence of the VH domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-4053; and

(e) the amino acid sequence of the VH domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-4054.

4. An antibody that immunospecifically binds G-protein Chemokine Receptor (CCR5) polypeptide, said antibody comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:76 or SEQ ID NO:72.

5. An antibody that immunospecifically binds G-protein Chemokine Receptor (CCR5) polypeptide, said antibody comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5861;

(b) the amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5860;

(c) the amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5859;

(d) the amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-4053; and

(e) the amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-4054.

6. An antibody that immunospecifically binds G-protein Chemokine Receptor (CCR5) polypeptide, said antibody comprising an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence selected from the group consisting of:

(a) SEQ ID NO:80;

(b) SEQ ID NO:78; and

(c) SEQ ID NO:74.

7. The antibody of claim 3, which further comprises an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of:

- (a) the amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5861;
- (b) the amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5860;
- (c) the amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-5859;
- (d) the amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-4053; and
- (e) the amino acid sequence of the VL domain of the antibody expressed by the hybridoma cell line deposited under ATCC Deposit No. PTA-4054.

8. The antibody of claim 4, which further comprises an amino acid sequence that is at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to an amino acid sequence selected from the group consisting of:

- (a) SEQ ID NO:80;
- (b) SEQ ID NO:78; and
- (c) SEQ ID NO:74.

9. The antibody of any one of claims 1 to 8, which further comprises an immunoglobulin heavy chain constant domain selected from the group consisting of:

- (a) an IgM constant domain;
- (b) an IgG constant domain; and
- (c) an IgA constant domain.

10. The antibody of claim 9 wherein the immunoglobulin heavy chain constant domain is a human immunoglobulin heavy chain constant domain.

11. The antibody of claim 10 wherein the human immunoglobulin heavy chain constant domain is selected from the group consisting of an IgG1 constant domain, an IgG2 constant domain, an IgG3 constant domain and an IgG4 constant domain.
12. The antibody of any one of claims 1 to 8, which further comprises an immunoglobulin light chain constant domain selected from the group consisting of:
 - (a) a kappa constant domain; and
 - (b) a lambda constant domain.
13. The antibody of claim 12 wherein the immunoglobulin light chain constant domain is a human immunoglobulin light chain constant domain.
14. The antibody of any one of claims 1 to 13, wherein said G-protein Chemokine Receptor (CCR5) polypeptide is selected from the group consisting of:
 - (a) the N-terminal extracellular region;
 - (b) extracellular loop 1;
 - (c) extracellular loop 2;
 - (d) extracellular loop 3; and
 - (e) the full-length CCR5 receptor.
15. The antibody of any one of claims 1 to 14, which immunospecifically binds to G-protein Chemokine Receptor (CCR5) in a Western blot or in an ELISA.
16. The antibody of any one of claims 1 to 15, wherein said antibody is selected from the group consisting of:
 - (a) a Fab fragment;
 - (b) a Fab' fragment;
 - (c) an F(ab')₂;
 - (d) an Fd;
 - (e) a single chain Fv;
 - (f) a disulfide linked Fv;
 - (g) a monoclonal antibody;
 - (h) a human antibody; and

(i) a humanized antibody.

17. The antibody of any one of claims 1 to 16, wherein said antibody has a dissociation constant (K_D) of 4×10^{-9} M or less.

18. The antibody of any one of claims 1 to 17, wherein said antibody has an off-rate selected from the group consisting of:

- (a) an off-rate of at least 10^{-3} /sec; and
- (b) an off-rate of at least 10^{-4} /sec.

19. The antibody of any one of claims 1 to 18, wherein said antibody is fused to a heterologous polypeptide.

20. The antibody of claim 19, wherein the heterologous polypeptide is human serum albumin.

21. The antibody of any one of claims 1 to 20, wherein said antibody is coupled or conjugated to a detectable label.

22. The antibody of claim 21, wherein the detectable label is a radioactive label.

23. The antibody of claim 22, wherein the radioactive label is ^{125}I , ^{131}I , ^{111}In , ^{90}Y , ^{99}Tc , ^{177}Lu , ^{166}Ho , or ^{153}Sm .

24. The antibody of claim 21, wherein the detectable label is an enzyme, a fluorescent label, a luminescent label, or a bioluminescent label.

25. The antibody of any one of claims 1 to 24, wherein the antibody is biotinylated.

26. The antibody of any one of claims 1 to 25, wherein said antibody is an antagonist of G-protein Chemokine Receptor (CCR5).

27. The antibody of claim 26, wherein said antibody has an activity selected from the group consisting of:

- (a) neutralization of G-protein Chemokine Receptor (CCR5);
- (b) inhibition of Eotaxin binding to G-protein Chemokine Receptor (CCR5) expressing cells;
- (c) inhibition of Eotaxin induced chemotaxis to G-protein Chemokine Receptor (CCR5) expressing cells;
- (d) inhibition of RANTES binding to G-protein Chemokine Receptor (CCR5) expressing cells;
- (e) inhibition of RANTES induced chemotaxis to G-protein Chemokine Receptor (CCR5) expressing cells;
- (f) inhibition of MCP-1 binding to G-protein Chemokine Receptor (CCR5) expressing cells;
- (g) inhibition of MCP-1 induced chemotaxis to G-protein Chemokine Receptor (CCR5) expressing cells;
- (h) inhibition of MCP-2 binding to G-protein Chemokine Receptor (CCR5) expressing cells;
- (i) inhibition of MCP-2 induced chemotaxis to G-protein Chemokine Receptor (CCR5) expressing cells;
- (j) inhibition of MCP-3 binding to G-protein Chemokine Receptor (CCR5) expressing cells;
- (k) inhibition of MCP-3 induced chemotaxis to G-protein Chemokine Receptor (CCR5) expressing cells;
- (l) inhibition of MIP-1alpha binding to G-protein Chemokine Receptor (CCR5) expressing cells;
- (m) inhibition of MIP-1alpha induced chemotaxis to G-protein Chemokine Receptor (CCR5) expressing cells;
- (n) inhibition of MIP-1beta binding to G-protein Chemokine Receptor (CCR5) expressing cells;
- (o) inhibition of MIP-1beta induced chemotaxis to G-protein Chemokine Receptor (CCR5) expressing cells;

- (p) downregulation of G-protein Chemokine Receptor (CCR5) expression; and
- (q) upregulation of G-protein Chemokine Receptor (CCR5) expression.

28. The antibody of any one of claims 1 to 25, wherein said antibody is an agonist of G-protein Chemokine Receptor (CCR5).

29. The antibody of any one of claims 1 to 25, wherein said antibody has an activity selected from the group consisting of:

- (a) inhibition of HIV binding to G-protein Chemokine Receptor (CCR5) expressing cells; and
- (b) inhibition of HIV infection of G-protein Chemokine Receptor (CCR5) expressing cells.

30. The antibody of any one of claims 1 to 29, wherein said antibody is conjugated to a therapeutic or cytotoxic agent.

31. The antibody of claim 30, wherein the therapeutic or cytotoxic agent is an antimetabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, a toxin, or an apoptotic agent.

32. An antibody that binds the same epitope on G-protein Chemokine Receptor (CCR5) as the antibody expressed by any one of the hybridoma cell lines selected from the group consisting of:

- (a) the hybridoma cell line deposited under ATCC Deposit No. PTA-5861;
- (b) the hybridoma cell line deposited under ATCC Deposit No. PTA-5860;
- (c) the hybridoma cell line deposited under ATCC Deposit No. PTA-5859;
- (d) the hybridoma cell line deposited under ATCC Deposit No. PTA-4053; and

(e) the hybridoma cell line deposited under ATCC Deposit No. PTA-4054.

33. An antibody that binds the same epitope on G-protein Chemokine Receptor (CCR5) as an antibody consisting of a first amino acid sequence and a second amino acid sequence, wherein said first and second amino acid sequences are selected from the group consisting of:

(a) a first amino acid sequence comprising the amino acid sequence of SEQ ID NO:76 and a second amino acid sequence comprising the amino acid sequence of SEQ ID NO:80;

(b) a first amino acid sequence comprising the amino acid sequence of SEQ ID NO:72 and a second amino acid sequence comprising the amino acid sequence of SEQ ID NO:74;

(c) a first amino acid sequence comprising the amino acid sequence of SEQ ID NO:72 and a second amino acid sequence comprising the amino acid sequence of SEQ ID NO:80; and

(d) a first amino acid sequence comprising the amino acid sequence of SEQ ID NO:76 and a second amino acid sequence comprising the amino acid sequence of SEQ ID NO:78.

34. The antibody of any one of claims 1 to 33, in a pharmaceutically acceptable carrier.

35. A polynucleotide encoding at least the VH domain or at least the VL domain of an antibody of any one of claims 1-8.

36. A vector comprising the polynucleotide of claim 35.

37. A host cell comprising the vector of claim 36 or the polynucleotide of claim 35.

38. A cell line engineered to express the antibody of any one of claims 1 to 20 and 32 or 33.

39. The cell line of claim 38, wherein the cells are NSO cells or CHO cells.
40. An antibody obtainable by expression from the cell line of claim 38 or 39.
41. A method of making an antibody comprising:
- (a) expressing the antibody encoded by the polynucleotide of claim 35;
- and
- (b) recovering said antibody.
42. An antibody obtainable by the method of claim 41.
43. Use of an antibody of any one of claims 1 to 34, 40 or 42 for the preparation of a pharmaceutical composition for the treatment or prevention of a disease or disorder selected from the group consisting of:
- (a) a disease or disorder associated with inflammation;
 - (b) a disease or disorder associated with defective or aberrant chemotaxis of immune cells;
 - (c) a disease or disorder associated with defective or aberrant T-cell antigen presenting cell interaction;
 - (d) an infectious disease;
 - (e) an autoimmune disease;
 - (f) Rheumatoid Arthritis;
 - (g) Host-versus-Graft disease;
 - (h) Graft-versus-Host disease;
 - (i) Crohn's disease;
 - (j) Wegner's granulomatosis;
 - (k) a neurodegenerative disorder;
 - (l) a viral infection;
 - (m) HIV infection;
 - (n) an early stage HIV infection;
 - (o) a cytomegalovirus infection;
 - (p) a poxvirus infection;

- (q) a *Pneumocystis carinii* infection;
- (r) Kaposi's sarcoma;
- (s) a disease or disorder associated with aberrant G-protein Chemokine Receptor (CCR5) expression;
- (t) a disease or disorder associated with the lack of G-protein Chemokine Receptor (CCR5) function;
- (u) a disease or disorder associated with aberrant G-protein Chemokine Receptor (CCR5) ligand expression; and
- (v) a disease or disorder associated with the lack of G-protein Chemokine Receptor (CCR5) ligand function.

44. The use of claim 43, wherein the pharmaceutical composition is for administration in a human.

45. The use of claim 43 or 44, wherein the pharmaceutical composition is for administration of the antibody in combination with a chemotherapeutic agent or an anti-retroviral agent.

46. The use of any one of claims 43 to 45, wherein the pharmaceutical composition further comprises an anti-retroviral agent.

47. A method of detecting expression of a G-protein Chemokine Receptor (CCR5) polypeptide comprising:

- (a) assaying the expression of a G-protein Chemokine Receptor (CCR5) polypeptide in a biological sample from an individual using the antibody of any one of claims 1 to 34, 40 or 42; and
- (b) comparing the level of a G-protein Chemokine Receptor (CCR5) polypeptide with a standard level of a G-protein Chemokine Receptor (CCR5) polypeptide.

48. A method of detecting, diagnosing, prognosing, or monitoring cancers or other hyperproliferative disorders comprising:

(a) assaying the expression of a G-protein Chemokine Receptor (CCR5) polypeptide in a biological sample from an individual using the antibody of any one of claims 1 to 34, 40 or 42; and

(b) comparing the level of a G-protein Chemokine Receptor (CCR5) polypeptide with a standard level of a G-protein Chemokine Receptor (CCR5) polypeptide.

49. A kit comprising the antibody of any one of claims 1 to 34, 40 or 42.

50. The kit of claim 49 further comprising a control antibody.

51. An isolated antibody or fragment thereof comprising a first amino acid sequence at least 95% identical to a second amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of a VHCDR1, VHCDR2 or VHCDR3 domain expressed by the hybridoma selected from the group consisting of:

- (i) ATCC Deposit No. PTA-5861;
- (ii) ATCC Deposit No. PTA-5860;
- (iii) ATCC Deposit No. PTA-5859;
- (iv) ATCC Deposit No. PTA-4053; or
- (v) ATCC Deposit No. PTA-4054;

and

(b) the amino acid sequence of a VLCDR1, VLCDR2 or VLCDR3 domain expressed by the hybridoma selected from the group consisting of:

- (i) ATCC Deposit No. PTA-5861;
- (ii) ATCC Deposit No. PTA-5860;
- (iii) ATCC Deposit No. PTA-5859;
- (iv) ATCC Deposit No. PTA-4053; or
- (v) ATCC Deposit No. PTA-4054;

wherein said antibody or fragment thereof immunospecifically binds G-protein Chemokine Receptor (CCR5).

52. An isolated antibody or fragment thereof comprising a first amino acid sequence at least 95% identical to a second amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of a VHCDR1, VHCDR2 or VHCDR3 domain comprising the amino acid sequence of SEQ ID NO:76 or SEQ ID NO:72;

and

(b) the amino acid sequence of a VLCDR1, VLCDR2 or VLCDR3 domain comprising the amino acid sequence selected from the group consisting of:

(i) SEQ ID NO:80;

(ii) SEQ ID NO:78; or

(iii) SEQ ID NO:74;

wherein said antibody or fragment thereof immunospecifically binds G-protein Chemokine Receptor (CCR5).

10 30 50
GTGAGATGGTGCTTTCATGAATTCCTCCCAACAAGAGCCAAGCTCTCCATCTAGTGGACAG
70 90 110
GGAAGCTAGCAGCAAACCTTCCCTTCACTACGAACTTCATTGCTTGGCCCAAAAGAGAG
130 150 170
TTAATTCAATGTAGACATCTATGTAGGCAATTAACCTATTGATGTATAAACAGTTT
190 210 230
GCATTCATGGAGGGCAACTAAATACATTCTAGGACTTTATAAAAGATCACTTTTTATTTA
250 270 290
TGCACAGGGTGAACAAGATGGATTATCAAGTGTCAAGTCCAATCTATGACATCAATTAT
M D Y Q V S S P I Y D I N Y
310 330 350
TATACATCGGAGCCCTGCCCAAAAATCAATGTGAAGCAAATCGCAGCCCGCCTCCTGCCT
Y T S E P C P K I N V K Q I A A R L L P
370 390 410
CCGCTCTACTCACTGGTGTTCATCTTTGGTTTTGTGGCAACATGCTGGTCATCCTCATC
P L Y S L V F I F G F V G N M L V I L I
430 450 470
CTGATAAACTGCCAAAGGCTGGAGAGCATGACTGACATCTACCTGCTCAACCTGGCCATC
L I N C Q R L E S M T D I Y L L N L A I
490 510 530
TCTGACCTGTTTTCTTCTTACTGTCCCCTTCTGGGCTCACTATGCTGCCGCCAGTGG
S D L F F L L T V P F W A H Y A A A Q W
550 570 590
GACTTTGGAATACAATGTGTCAACTCTTGACAGGGCTCTATTTTATAGCCTTCTTCTCT
D F G N T M C Q L L T G L Y F I G F F S
610 630 650
GGAATCTTCTTCATCATCCTCCTGACAATCGATAGGTACCTGGCTATCGTCCATGCTGTG
G I F F I I L L T I D R Y L A I V H A V
670 690 710
TTTGCTTTAAAGCCAGGACGGTCACCTTTGGGGTGGTGACAAGTGTGATCACTTGGGTG
F A L K A R T V T F G V V T S V I T W V
730 750 770
GTGGCTGTGTTTGGCTCTCTCCCAGGAATCATCTTTACCAGATCTCAAAAAGAAGGTCTT
V A V F A S L P G I I F T R S Q K E G L
790 810 830
CATTACACCTGCAGCTCTCATTTTCCATACAGTCAGTATCAATTCTGGAAGAATTTCCAG
H Y T C S S H F P Y S Q Y Q F W K N F Q
850 870 890
ACATTAAAGATAGTCATCTTGGGGCTGGTCCTGCCGCTGCTTGTATGGTCATCTGCTAC
T L K I V I L G L V L P L L V M V I C Y
910 930 950
TCGGGAATCCTAAAACTCTGCTTCGGTGTGGAATGAGAAGAAGAGGCACAGGGCTGTG
S G I L K T L L R C R N E K K R H R A V

FIG.1A

970 990 1010
AGGCTTATCTTCACCATCATGATTGTTTATTTCTCTTCTGGGCTCCCTACAACATTGTC
R L I F T I M I V Y F L F W A P Y N I V
1030 1050 1070
CTTCTCCTGAACACCTTCCAGGAATTCTTTGGCCTGAATAATTGCAGTAGCTCTAACAGG
L L L N T F Q E F F G L N N C S S S N R
1090 1110 1130
TTGGACCAAGCTATGCAGGTGACAGAGACTCTTGGGATGACGCACTGCTGCATCAACCCC
L D Q A M Q V T E T L G M T H C C I N P
1150 1170 1190
ATCATCTATGCCTTTGTGCGGGAGAAGTTCAGAACTACCTCTTAGTCTTCTTCCAAAAG
I I Y A F V G E K F R N Y L L V F F Q K
1210 1230 1250
CACATTGCCAAACGCTTCTGCAAATGCTGTTCTATTTTCCAGCAAGAGGCTCCCGAGCGA
H I A K R F C K C C S I F Q Q E A P E R
1270 1290 1310
GCAAGCTCAGTTTACACCCGATCCACTGGGGAGCAGGAAATATCTGTGGGCTTGTGACAC
A S S V Y T R S T G E Q E I S V G L *
1330 1350 1370
GGAAGTCAAGTGGGCTGGTGACCCAGTCAGAGTTGTGCACATGGCTTAGTTTTTCATACACA
1390 1410
GCCTGGGCTGGGGGTGGGGTGAAGAGGTCTTTT

FIG.1B

4	QVSSPIYDINYYTSEPCPKINVKQIAARLLPPLYSLVFIFGFVGNMLVIL	53
18	EEVTTFFDYDY..GAPCHKFDVKQIGAQLLPPLYSLVFIFGFVGNMLVVL	65
54	ILINCQRLESMTDIYLLNLAISDLFFLLTVPFWAHYAAAQWDFGNTMCQL	103
66	ILINCKKLKCLTDIYLLNLAISDLLFLITLPLWAHSAANEWVFGNAMCKL	115
104	LTGLYFIFGFSGIFFIILLTIDRYLAIVHAVFALKARTVTFGVVTSVITW	153
116	FTGLYHIGYFGGIFFIILLTIDRYLAIVHAVFALKARTVTFGVVTSVITW	165
154	VVAVFASLPGIIFTRSQKEGLHYTCSSHFPYSQYQFWKNFQTLKIVILGL	203
166	LVAVFASVPGIIFTKCQKEDSVYVCGPYFPRG....WNNFHTIMRNILGL	211
204	VLPLLVMVICYSGILKTLLRCRNEKKRHRÄVRLIFTIMIVYFLFWAPYNI	253
212	VLPLLIMVICYSGILKTLLRCRNEKKRHRÄVRVIFTIMIVYFLFWTPYNI	261
254	VLLLNTFQEFGGLNNCSSSNRLDQAMQVTE TLGMTHCCINPIIYAFVGEK	303
262	VILLNTFQEFGGLSNCESTSQLDQATQVTE TLGMTHCCINPIIYAFVGEK	311
304	FRNYLLVFFQKHI AKRFCKCCSIFQQEAPERASSVYTRS...TGEQEISV	350
312	FRSLFHIALGCRIA.PLQKPVCGGPGVRPGKNVKVTTQGLLDGRGKGKSI	360
351	G 351	
361	G 361	

FIG. 2

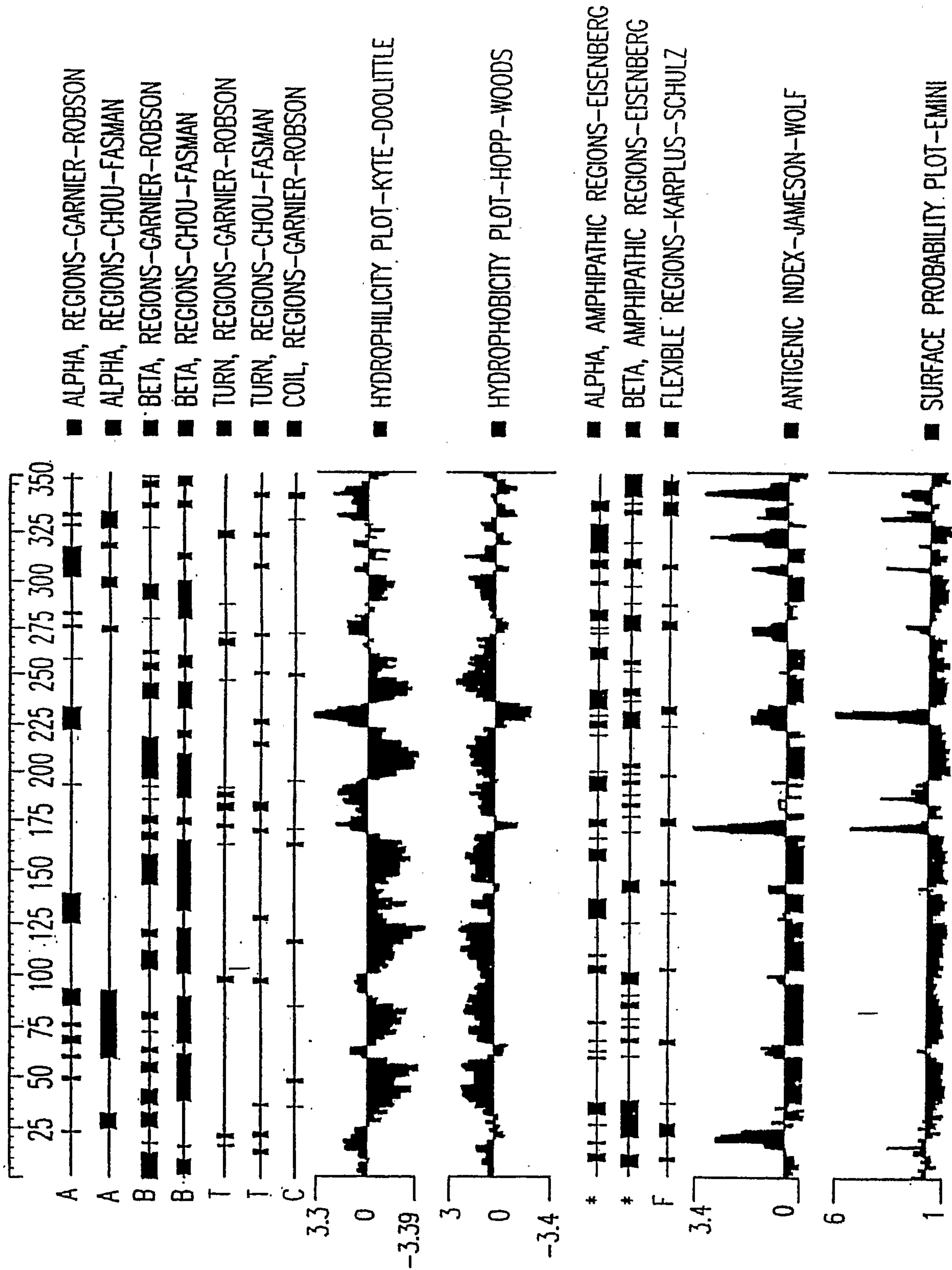


FIG.3

36F11 VH

1 CAG GTG CAG TTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG AGA CTC 60
 1 Q V Q L V E S G G G V V Q P G R S L R L 20

CDR1

61 TCC TGT GTA GCC TCT GGA TTC ACC TTA AGT GGC TAT GGC CTG CAC TGG GTC CGC CAG GCT 120
 21 S C V A S G F T L S G Y G L H W V R Q A 40

CDR2

121 CCA GGC AAG GGG CTG GAG TGG GTG GCA CTT ATA TCA TAT GAT GGA AGT AAT AGA TAC TAT 180
 41 P G K G L E W V A L I S Y D G S N R Y Y 60

181 GGA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC GAT TCC AAG AAC ACG CTA TAT 240
 61 G D S V K G R F T I S R D D S K N T L Y 80

241 CTG CAA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT GCG AGA GGA CGA 300
 81 L Q M N S L R A E D T A V Y Y C A R G R 100

CDR3

301 AAC GAT TTT TGG AGT GGT TAT TAT ACG GCC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG 360
 101 N D F W S G Y Y T A G M D V W G Q G T T 120

361 GTC ACC GTC TCC TCA 375
 121 V T V S S 125

36F11 VL

1 GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA AGA GAC AGA GTC TCC 60
 1 D I Q M T Q S P S S L S A S V R D R V S 20

CDR1

61 ATC ACT TGC CAG GCG AGT CAG GAC ATT AGC AAC TAT TTA AAT TGG TAT CAG CAG AAA CCA 120
 21 I T C Q A S Q D I S N Y L N W Y Q Q K P 40

CDR2

121 GGG AAA GCC CCT AAA CTC CTG ATC TAC GAT GCA TCC GAT TTG GAA ACA GGG GTC CCA TCA 180
 41 G K A P K L L I Y D A S D L E T G V P S 60

181 AGG TTC AGT GGA AGT GGA TCT GGG ACA GAT TTT ACT TTC ACC ATC AGC AGC CTG CAG CCT 240
 61 R F S G S G S G T D F T F T I S S L Q P 80

CDR3

241 GAA GAT ATT GCA ACA TAT TAC TGT CAA CAG TAT GAT AAT TTT CCA TTC ACT TTC GGC CCT 300
 81 E D I A T Y Y C Q Q Y D N F P F T F G P 100

301 GGG ACC AAA GTG GAT ATC AAA CGT 324
 101 G T K V D I K R 108

FIG. 4

43E2 VH

1 CAG GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG AGG TCC CTG AGA CTC 60
 1 Q V Q L V E S G G G V V Q P G R S L R L 20

CDR1

61 TCC TGT GCA GCC TCT GGA TTC ACC TTA AGT GGC TAT GGC ATG CAC TGG GTC CGC CAG GCT 120
 21 S C A A S G F T L S G Y G M H W V R Q A 40

CDR2

121 CCA GGC AAG GGG CTG GAG TGG GTG TCA CTT ATA TCA TAT GAT GGA AGT AAT AAA TAC TAT 180
 41 P G K G L E W V S L I S Y D G S N K Y Y 60

181 GCA GAC TCC GTG AAG GGC CGA TTC ACC ATC TCC AGA GAC GAT TCC AAG AAC ACG CTG TAT 240
 61 A D S V K G R F T I S R D D S K N T L Y 80

241 CTG CGA ATG AAC AGC CTG AGA GCT GAG GAC ACG GCT GTG TAT TAC TGT GCG AGA GGA CGA 300
 81 L R M N S L R A E D T A V Y Y C A R G R 100

CDR3

301 AAC GAT TTT TGG AGT GGT TAT TAT ACG GCC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG 360
 101 N D F W S G Y Y T A G M D V W G Q G T T 120

361 GTC ACC GTC TCC TCA 375
 121 V T V S S 125

43E2 VL

1 GAC ATC CAG ATG ACC CAG TCT CCA TCC TCC CTG TCT GCA TCT GTA GGA GAC AGA GTC ACC 60
 1 D I Q M T Q S P S S L S A S V G D R V T 20

CDR1

61 ATC ACT TGC CAG GCG AGT CAG GAC ATT AGT AAT TAT TTA AAT TGG TAT CAG CAG AAA CCA 120
 21 I T C Q A S Q D I S N Y L N W Y Q Q K P 40

CDR2

121 GGG AAA GCC CCT AAG CTC CTG ATC TAC GAT GCA TCC AAT TTG GAA ACA GGG GTC CCA TCA 180
 41 G K A P K L L I Y D A S N L E T G V P S 60

181 AGG TTC AGT GGA AGT GGA TCT GGG ACA GAT TTT ACT TTC ACC ATC AAC AGC CTG CAG CCT 240
 61 R F S G S G S G T D F T F T I N S L Q P 80

CDR3

241 GAA GAT ATT GCA ACA TAT TAC TGT CAA CAG TAT GAT AAT TTC CCA TTC ACT TTC GGC CCT 300
 81 E D I A T Y Y C Q Q Y D N F P F T F G P 100

301 GGG ACC AAA GTG GAT ATC AAA CGT 324
 101 G T K V D I K R 108

FIG. 5

AK38181 VL

1	GAC	ATC	CAG	ATG	ACC	CAG	TCT	CCA	TCC	TCC	CTG	TCT	GCA	TCT	GTA	GGA	GAC	AGA	GTC	ACC	60
1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T	20
CDR1																					
61	ATC	ACT	TGC	CAG	GCG	AGT	CAG	GGC	ATT	CGC	AAA	TAT	TTA	AAT	TGG	TAT	CAG	CAA	AAA	CCA	120
21	I	T	C	Q	A	S	Q	G	I	R	K	Y	L	N	W	Y	Q	Q	K	P	40
CDR2																					
121	GGG	AAA	GTC	CCT	AAG	CTC	CTG	ATC	TAC	GAT	GCA	TCC	AAT	TTG	GAA	ACA	GGG	GTC	CCA	TCA	180
41	G	K	V	P	K	L	L	I	Y	D	A	S	N	L	E	T	G	V	P	S	60
181	AGG	TTC	AGT	GGA	AGT	GGA	TCA	GGG	ACA	GAT	TTT	ACT	TTC	GCC	ATC	AGC	AGC	CTG	CAG	CCG	240
61	R	F	S	G	S	G	S	G	T	D	F	T	F	A	I	S	S	L	Q	P	80
CDR3																					
241	GAA	GAT	ACT	GCA	ACA	TAT	TAC	TGT	CAA	CAA	TAT	GAT	GAT	TTC	CCC	TTC	ACC	TTC	GGC	CAG	300
81	E	D	T	A	T	Y	Y	C	Q	Q	Y	D	D	F	P	F	T	F	G	Q	100
301	GGG	ACA	CGA	CTG	GAG	ATT	AAA	CGT	324												
101	G	T	R	L	E	I	K	R	108												

FIG. 6

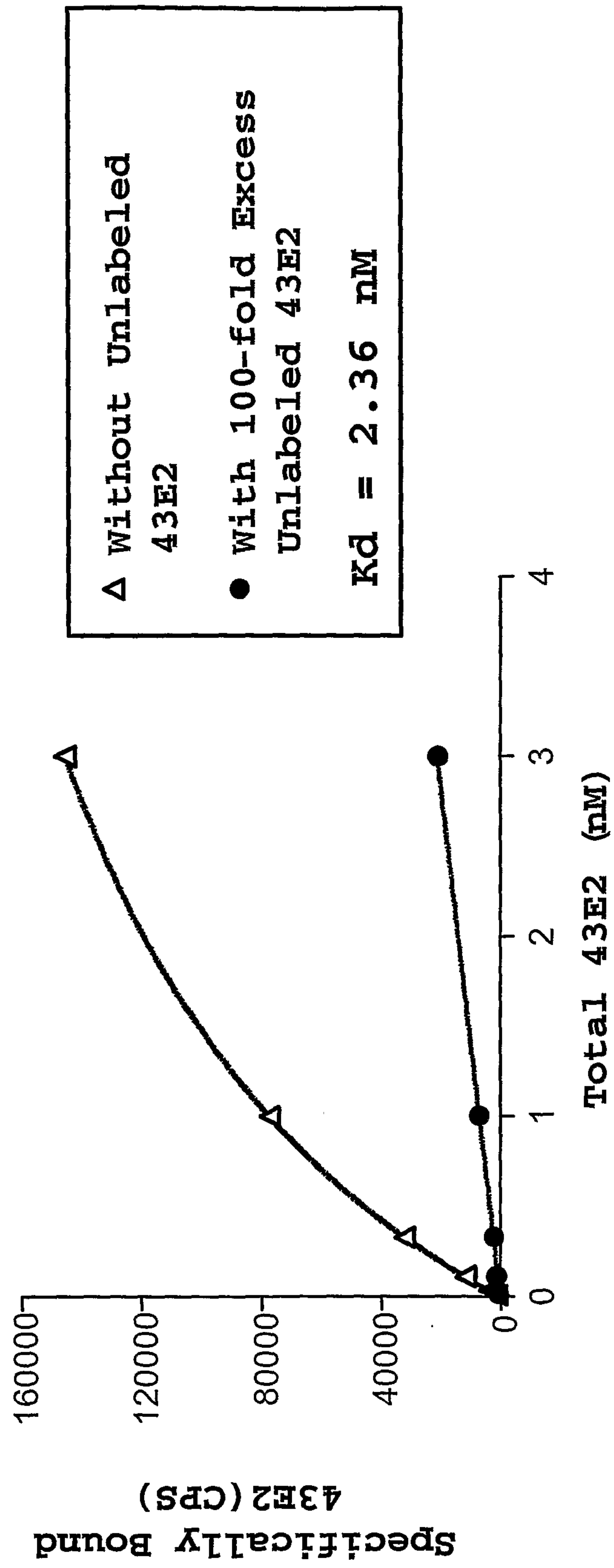


FIG. 7A

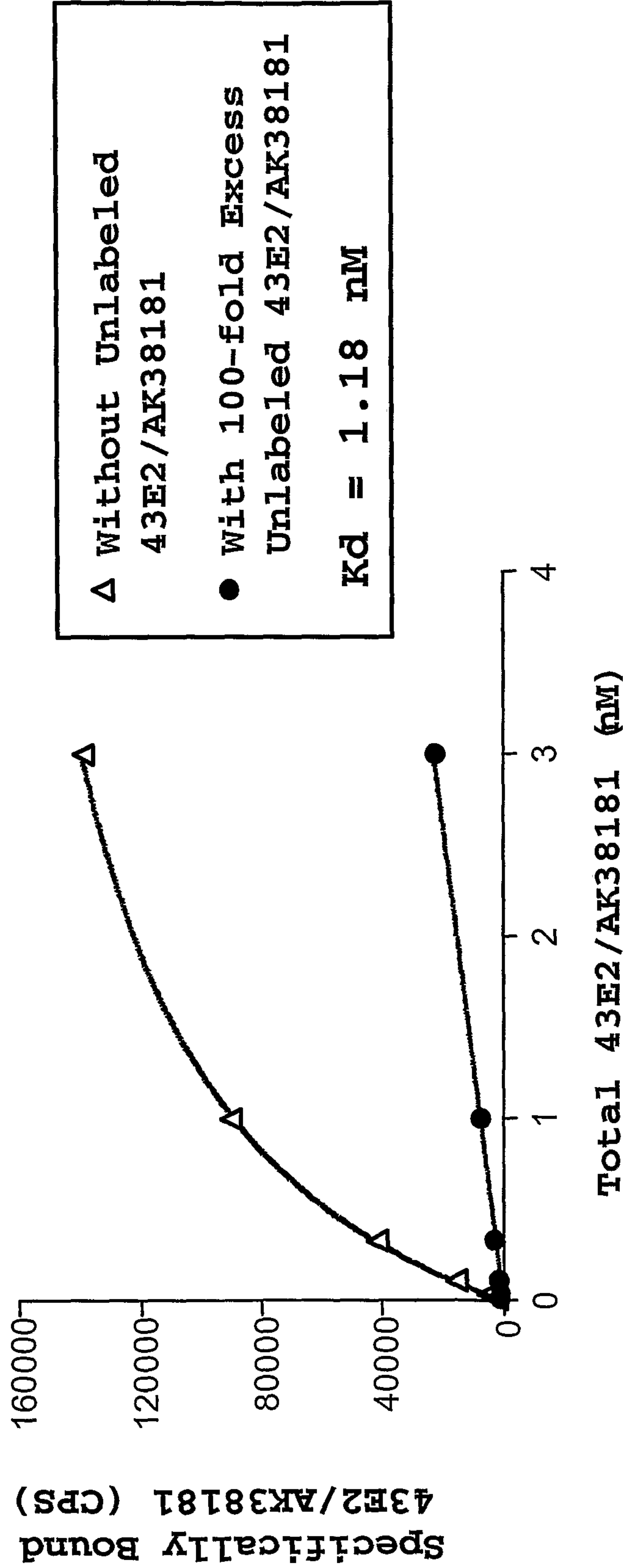
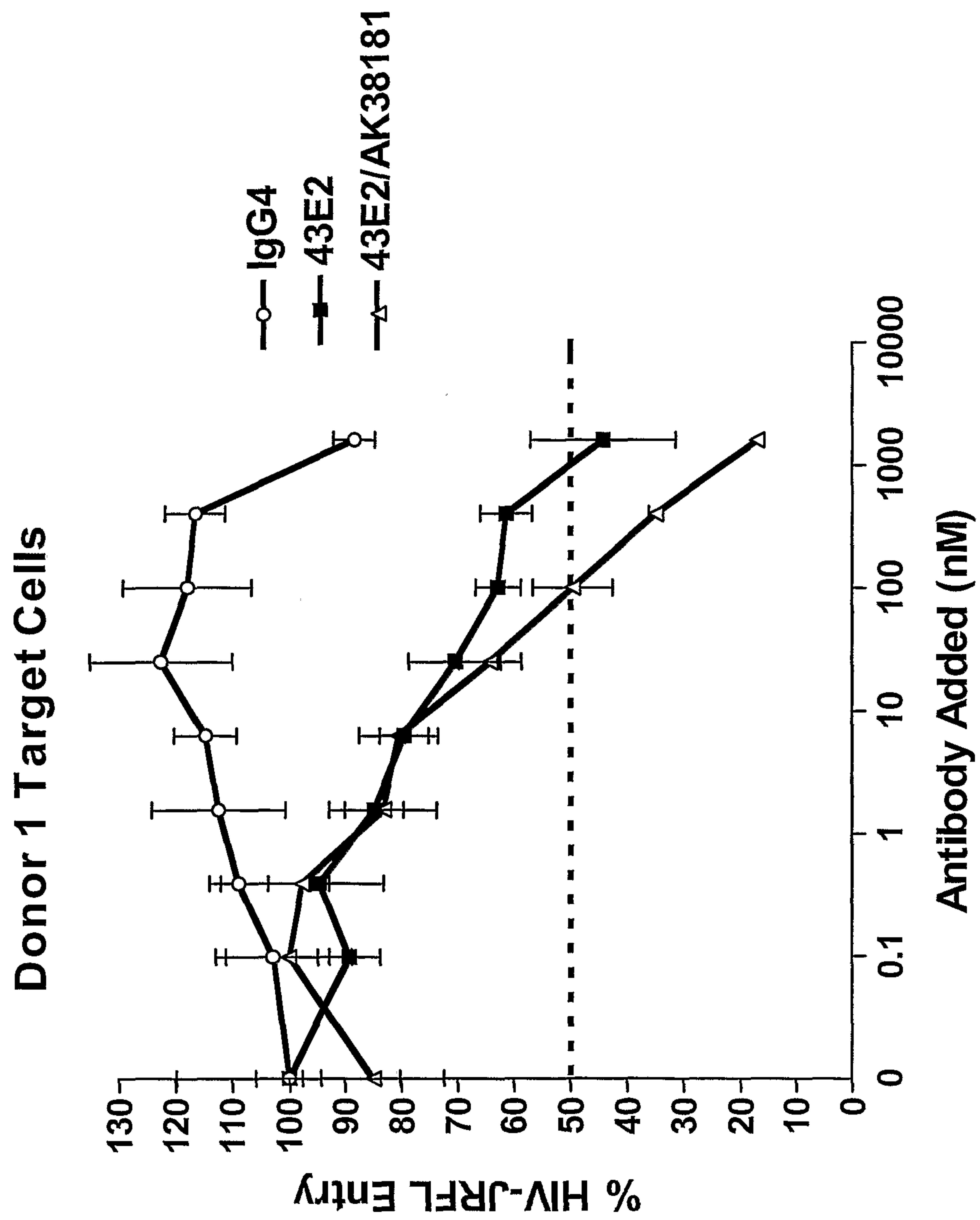


FIG. 7B

**FIG. 8A**

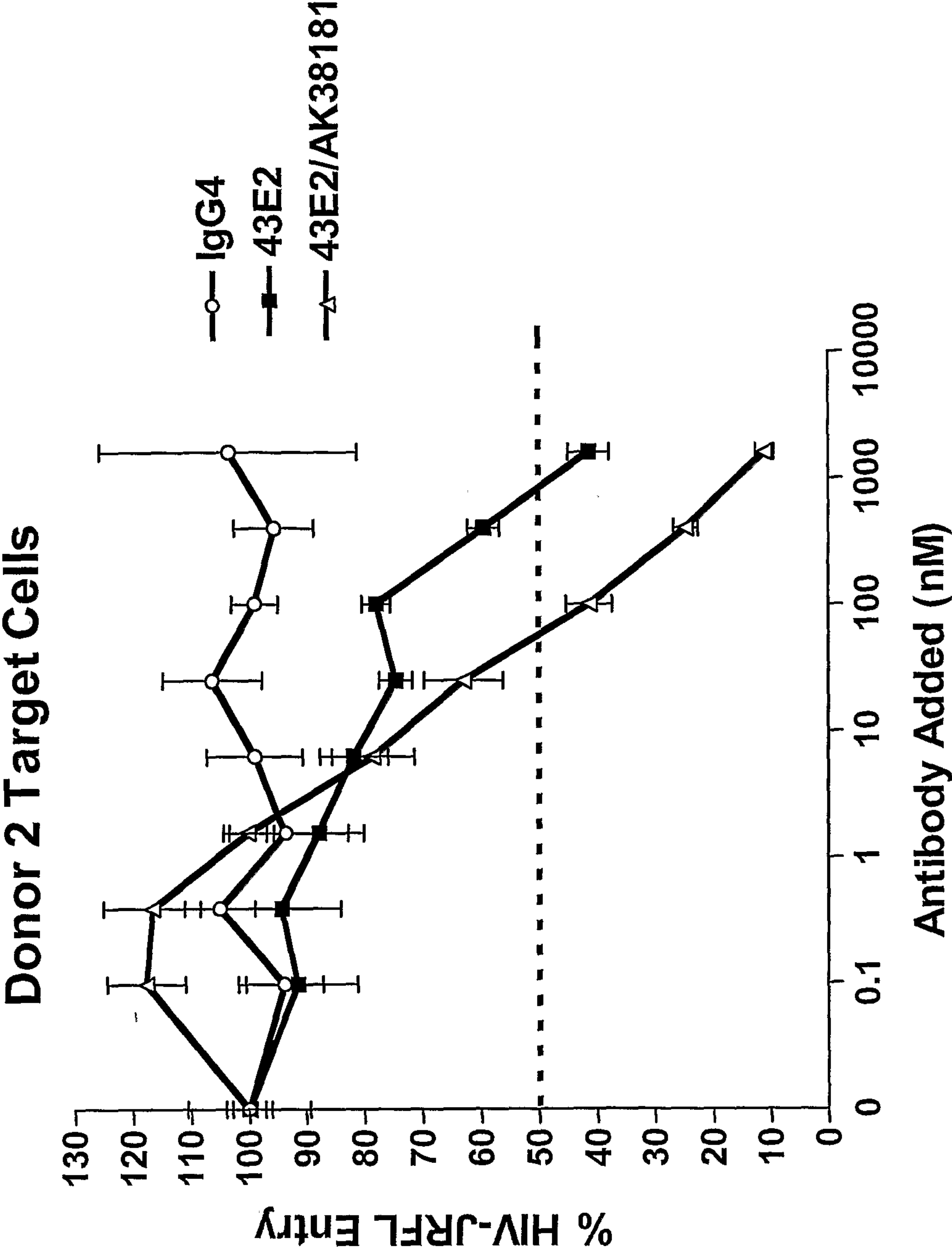


FIG. 8B

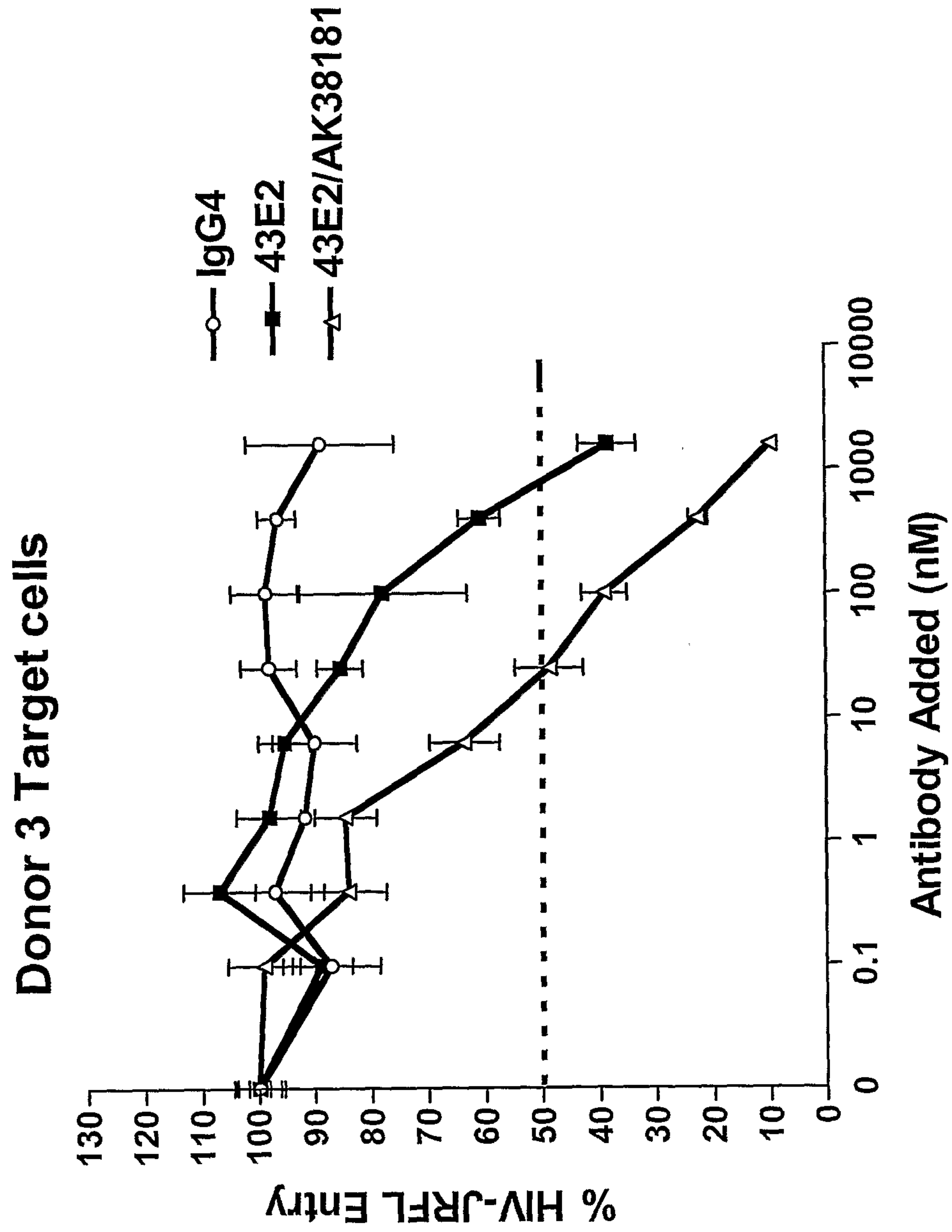


FIG. 8C