

ANTIBODIES TO CCR2

CROSS-REFERENCE TO RELATED PATENTS AND PATENT APPLICATIONS

5 **[001]** This application claims the benefit of U.S. Provisional Patent Application No. 61/189,357, filed August 18, 2008, which is incorporated by reference herein in its entirety.

JOINT RESEARCH AGREEMENT

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[002] The disclosure and claims herein were made as a result of activities undertaken within the scope of a joint research agreement between Pfizer Inc. and Abgenix Inc. that was in effect on or before the date the claimed subject matter was made.

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BACKGROUND

[003] Leukocyte infiltration into inflammatory sites is believed to be regulated by 8-10 kD proteins known as chemokines. These chemokines are classified into
20 four groups, depending on the spacing of their N-terminal cysteine residues, designated CC, CXC, XC and CX3C. Chemokines can mediate a range of pro-inflammatory effects on leukocytes, such as triggering of chemotaxis, degranulation, synthesis of lipid mediators, and integrin activation (Oppenheim, J. J. *et al.*, *Annu. Rev. Immunol.*, 9:617-648 (1991); Baggiolini, M., *et al.*, *Adv. Immunol.*, 55:97-179 (1994); Miller, M. D. and Krangel, M. S., *Crit. Rev. Immunol.*,
25 12:17-46 (1992)).

[004] One chemokine, Monocyte Chemotactic Protein 1 (MCP-1), also known as CCL2, acts upon monocytes, lymphocytes and dendritic cells, to induce chemotaxis, granule release, respiratory burst and cytokine release. Studies have
30 suggested that MCP-1 is implicated in the pathology of diseases such as rheumatoid arthritis, atherosclerosis, granulomatous diseases, chronic obstructive pulmonary disease (COPD), obesity/diabetes, neuropathic pain, cancer, and

multiple sclerosis (Koch, *J. Clin. Invest.* 90:772-79 (1992); Hosaka *et al.*, *Clin. Exp. Immunol.* 97:451-457 (1994); Schwartz *et al.*, *Am. J. Cardiol.* 71(6):9B-14B (1993); Schimmer *et al.*, *J. Immunol.* 160:1466-1471 (1998); Flory *et al.*, *Lab. Invest.* 69:396-404 (1993); Gong *et al.*, *J. Exp. Med.* 186:131-137 (1997); Salcedo *et al. Blood* 96(1) 34-40 (2000); Bracke *et al.*, *Inflammation & Allergy – Drug Targets* 6: 75-79 (2007); Chung *Current Drug Targets – Inflammation & Allergy* 4: 619-625 (2005).

[005] CCR2 is a seven-transmembrane domain G-protein coupled chemotactic receptor which binds MCP-1 as well as other chemokines including CCL8 (MCP-2), CCL7 (MCP-3) and CCL13 (MCP-4) (Charo, I. F., *et al.*, *Proc. Natl. Acad. Sci. USA* 91:2752-2756 (1994); Myers, S. J., *et al.*, *J. Biol. Chem.* 270:5786-5792 (1995); Gong *et al.*, *J. Biol. Chem.* 272:11682-11685 (1997); Garcia-Zepeda *et al.*, *J. Immunol.* 157:5613-5626 (1996)). CCR2 is also known as CMKBR2 and CKR2. Two alternatively-spliced forms of the CCR2, CCR2A and CCR2B, have been cloned which differ in their C-termini (Wong *et al.* (1997) *J. Biol. Chem.* 272:1038-1045). In signaling studies, both CCR2A and CCR2B mediate agonist-dependent calcium mobilization and adenylyl cyclase inhibition. CCR2 is expressed on monocytes, T cells, and dendritic cells, and interacts with chemokines secreted by endothelial cells, monocytes, and synovial fibroblasts.

[006] The biological role of CCR2 has been probed through the use of CCR2 knockout mice (Boring *et al.*, *J Clin Invest.* 100(10):2552-61 (1997); Boring *et al.*, *Nature* 394(6696):894-7 (1998); De Paolo *et al.*, *J Immunol.* 171(7):3560-7 (2003); Gaupp *et al.*, *Am J Pathol.* 162(1):139-50(2003)). CCR2-/- mice have significant defects in both delayed-type hypersensitivity responses and production of Th1-type cytokines, and are generally less susceptible to developing experimental autoimmune encephalomyelitis (EAE). In addition to modulating immune responses, CCR2 is a co-receptor for HIV (Connor *et al.*, *J. Exp. Med.* 185:621-628 (1997); Frade *et al.*, *J Clin Invest.* 100(3):497-502(1997)).

[007] Due to the involvement of MCP-1 and its receptor CCR2 in undesirable immune responses, CCR2 antagonists may be promising therapeutic agents. However, few CCR2 antagonists have been described (see Ogilvie *et al.*, *Blood* 97(7):1920-4 (2001)). Thus, there is a need for novel and improved compositions that will bind CCR2 and block CCR2 signaling mediated by its ligand.

SUMMARY

[008] Provided are isolated antibodies, or antigen-binding portions thereof, that specifically bind CCR2, particularly human CCR2, and may act as a CCR2 antagonist, and compositions comprising said antibodies or portions. Included are
5 antibodies or antigen-binding portions that bind to CCR2 at an epitope other than the N-terminal portion or the third loop of CCR2. Such antibodies may bind to the first and/or second extracellular loops of CCR2.

[009] Compositions comprising (i) the heavy and/or light chain, the variable domains thereof, or antigen-binding portions thereof, of said anti-CCR2 antibody,
10 or nucleic acid molecules encoding them; and (ii) a pharmaceutically acceptable carrier are provided. The compositions may further comprise another component, such as a therapeutic agent or a diagnostic agent.

[0010] Diagnostic and therapeutic methods are also provided. Similarly, anti-CCR2 antibodies and portions thereof for the manufacture of medicaments to
15 treat inflammatory and non-inflammatory disorders are provided.

[0011] Provided are vectors and host cells comprising the nucleic acid molecules, as well as methods of recombinantly producing the polypeptides encoded by the nucleic acid molecules. Isolated cell lines that produces an anti-CCR2 antibody or an antigen-binding portion thereof are also provided.

20 BRIEF DESCRIPTION OF THE DRAWINGS

[0012] Figures 1A, 1B, & 1C are graphs showing the binding of a CCR2 antibody to cells as assayed by FACS analysis. Figure 1A is a graph illustrating binding of the AF-488 (ALEXA FLUOR® 488, Invitrogen) conjugated CCR2 antibody 4.40 A68G S230P to human whole blood monocytes compared to a KLH control
25 antibody as assayed by FACS analysis. Figure 1B is a graph illustrating binding of AF-488 conjugated CCR2 antibody 4.40 A68G S230P to 300-19 cells expressing human CCR2 as assayed by FACS analysis. Figure 1C is a graph illustrating the binding of different concentrations of 4.40 A68G S230P antibody to CCR2 transfected 300-19 cells as detected with anti-human PE.

30 [0013] Figure 2 illustrates the dose related binding of 4.40 A68G S230P antibody to CCR2 transfected 300-19 cells in a saturation binding assay.

[0014] Figure 3 illustrates the ability of the 4.40 A68G S230P antibody to inhibit the chemotaxis of THP-1 cells in response to the CCR2 ligand MCP-1 but not in response to the CCR1/CCR5 ligand MIP-1a.

5 [0015] Figure 4 illustrates the ability of the 4.40 A68G S230P antibody to inhibit the chemotaxis of primary human monocytes in response to MCP-1.

[0016] Figure 5 shows the plasmid map of a retroviral vector for expression of the CCR1/CCR2 chimeras.

10 [0017] Figures 6A & 6B illustrates the binding of 4.40 A68G S230P antibody to 300-19 cells expressing a chimeric receptor consisting of only the extracellular 1st and 2nd loops of CCR2 and the N terminus and third loop of CCR1. Figure 6C depicts a saturation binding assay of 4.40 A68G S230P to the chimeric receptor transfected 300-19 cells as measured by FACS analysis.

15 [0018] Figure 7 shows saturation binding analysis (3hr saturation curve) of the 4.40 A68G S230P antibody on (A) 300-19 control cells; (B) transfected 300-19 cells expressing full length CCR2; (C) transfected 300-19 cells expressing flag-tagged (M1) MRRR chimera [N terminus of CCR2 (M) flag tagged to ensure receptor expression and the loop regions of CCR1 (R)]; (D) transfected 300-19 cells expressing flag-tagged (M1) RRRM chimera [N terminus and 1st and 2nd loop of CCR1 (R) flag tagged to ensure receptor expression and the 3rd loop of CCR2 (M)]; and (E) transfected 300-19 cells expressing flag-tagged (M1) RMMR chimera [N terminus and 3rd loop of CCR1 (R) flag tagged to ensure receptor expression and the 1st and 2nd loop of CCR2 (M)].

[0019] Figure 8 shows binding of the 4.40 A68G S230P antibody to either loop 2 or loop 3 peptide regions of CCR2 as assessed in a capture ELISA.

25 [0020] Figure 9 shows two graphs illustrating the FACS immunostaining of 300-19 cells expressing recombinant CCR5, with either the AF-488 conjugated 4.40.3 A68G S230P antibody (panel A) or with an anti-CCR5 antibody (panel B).

[0021] Figure 10 illustrates the inhibition of MCP-1 induced activity by 4.40 A68G S230P antibody as assessed by calcium mobilization on CCR2 transfected 300-19 cells.

[0022] Figure 11 demonstrates the inhibition of MCP-3 induced chemotaxis of CCR2 transfected 300-19 cells by the 4.40 A68G S230P antibody.

[0023] Figure 12 illustrates the inhibition of human monocyte actin polymerization in response to MCP-1 in whole blood by the 4.40 A68G S230P antibody.

[0024] Figure 13 illustrates the inhibition of monocyte actin polymerization in female cynomolgus monkey response to MCP-1 in whole blood by the 4.40 A68G S230P antibody.

[0025] Figure 14 shows the dose-dependent inhibition of collagen 1 mRNA synthesis in the hHSC cell line, LI90, by the 4.40 A68G S230P antibody.

[0026] Figure 15 demonstrates the dose-dependent inhibition of pERK phosphorylation in human whole blood at 10 nM MCP-1 by the 4.40 A68G S230P antibody.

[0027] Figure 16 shows the decrease of plasma alanine transaminase (ALT) and aspartate aminotransferase (AST) activities in human CCR2 knock-in mice by the 4.40 A68G S230P antibody 24 hours after a single ConA injection.

[0028] Figures 17A-17D show an alignment of the germline amino acid sequences of the heavy and light chain variable regions compared to the respective 4.22.3, 4.40.2, 4.39.3 and 4.9.2 antibody heavy and light chain variable regions (only mistakes are shown for the 4.22.3, 4.40.2, 4.39.3 and 4.9.2 antibody). The CDRs are underlined and mismatched gap(s) are indicated by a pound sign (#).

DETAILED DESCRIPTION

Definitions and General Techniques

[0029] Unless otherwise defined herein, scientific and technical terms used herein shall have the meanings that are commonly understood by those of ordinary skill in the art. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. All publications and other references mentioned herein are incorporated by reference in their entirety. Generally, nomenclature used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics, protein and nucleic acid chemistry, and hybridization described herein are those well known and commonly used in the art. In case of conflict, the present specification, including definitions, will control.

[0030] The methods and techniques are generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. See, e.g., Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, second ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989); Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates (1992); and Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1990), all of which are incorporated herein by reference. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclature used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

[0031] The following terms, unless otherwise indicated, shall be understood to have the following meanings:

[0032] The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

[0033] The term "isolated protein," "isolated polypeptide" or "isolated antibody" is a protein, polypeptide or antibody that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is free of other proteins from the same species, (3) is expressed by a cell from a different species, or (4) does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally-associated components by isolation, using protein purification techniques well known in the art.

[0034] Examples of isolated antibodies include an anti-CCR2 antibody that has been affinity purified using CCR2 or a portion thereof, an anti-CCR2 antibody that has been synthesized by a hybridoma or other cell line *in vitro*, and a human anti-CCR2 antibody derived from a transgenic mouse.

5 [0035] A protein or polypeptide is "substantially pure," "substantially homogeneous," or "substantially purified" when at least about 60 to 75% of a sample exhibits a single species of polypeptide. The polypeptide or protein may be monomeric or multimeric. A substantially pure polypeptide or protein will typically comprise about 50%, 60%, 70%, 80% or 90% W/W of a protein sample,
10 more usually about 95%, and may be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For certain purposes, higher resolution may be provided by using HPLC or other
15 means well known in the art for purification.

[0036] The term "antibody analog" as used herein refers to an antibody that comprises a segment that has substantial identity to a portion of an amino acid sequence and that has at least one of the following properties: (1) specific binding to CCR2 under suitable binding conditions, (2) ability to inhibit at least one
20 biological activity of CCR2. Typically, antibody analogs comprise a conservative amino acid substitution (or insertion or deletion) with respect to the native sequence. Analogs typically are at least 20 or 25 amino acids long, at least 50, 60, 70, 80, 90, 100, 150 or 200 amino acids long or longer, and can often be as long as a full-length heavy chains or light chains of the antibodies. Some cases
25 include antibody analogs with 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 substitutions from the germline amino acid sequence.

[0037] In certain cases, amino acid substitutions to an anti-CCR2 antibody or antigen-binding portion thereof are those which: (1) reduce susceptibility to proteolysis, (2) reduce susceptibility to oxidation, (3) alter binding affinity for
30 forming protein complexes, (4) add or remove glycosylation sites and (5) confer or modify other physicochemical or functional properties of such analogs, but still retain specific binding to CCR2. Analogs can include various muteins of a sequence other than the normally-occurring peptide sequence. For example,

single or multiple amino acid substitutions, such as conservative amino acid substitutions, may be made in the normally-occurring sequence in a portion of the polypeptide outside the domain(s) forming intermolecular contacts. A conservative amino acid substitution should not substantially change the structural characteristics of the parent sequence; e.g., a replacement amino acid should not alter the anti-parallel β -sheet that makes up the immunoglobulin binding domain that occurs in the parent sequence, or disrupt other types of secondary structure that characterizes the parent sequence. In general, glycine and proline would not be used in an anti-parallel β -sheet. Examples of art-recognized polypeptide secondary and tertiary structures are described in *Proteins, Structures and Molecular Principles* (Creighton, Ed., W. H. Freeman and Company, New York (1984)); *Introduction to Protein Structure* (C. Branden and J. Tooze, eds., Garland Publishing, New York, N.Y. (1991)); and Thornton *et al.*, *Nature* 354:105 (1991), incorporated herein by reference.

[0038] Where an "antibody" is referred to herein, it is normally understood that an antigen-binding portion thereof may also be used. An antigen-binding portion competes with the intact antibody for specific binding. See generally, *Fundamental Immunology*, Ch. 7 (Paul, W., ed., second ed. Raven Press, N.Y. (1989)) (incorporated by reference in its entirety for all purposes). Antigen-binding portions may be produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. In some cases, antigen-binding portions include Fab, Fab', F(ab')₂, Fd, Fv, dAb, and complementarity determining region (CDR) fragments, single-chain antibodies (e.g., scFv), chimeric antibodies, diabodies and polypeptides that contain at least a portion of an antibody that is sufficient to confer specific antigen binding to the polypeptides.

[0039] From N-terminus to C-terminus, both the mature light and heavy chain variable domains of an antibody comprise the regions FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain herein is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)); Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987); or Chothia *et al.*, *Nature* 342:878-883 (1989).

[0040] As used herein, an antibody that is referred to by number is the same as a monoclonal antibody that is obtained from the hybridoma of the same number. For example, monoclonal antibody 4.40 is the same antibody as one obtained from hybridoma 4.40, or a subclone thereof. Sequential subclones are designated
5 for example 4.40.1, 4.40.2, and 4.40.3, and have substantially the same sequences and functionality.

[0041] As used herein, an Fd fragment means an antibody fragment that consists of the V_H and C_H1 domains; an Fv fragment consists of the V_L and V_H domains of a single arm of an antibody; and a dAb fragment (Ward *et al.*, *Nature* 341:544-546
10 (1989)) consists of a V_H domain.

[0042] In some cases, the antibody is a single-chain antibody (e.g., scFv) in which V_L and V_H domains are paired to form a monovalent molecules via a synthetic linker that enables them to be made as a single polypeptide chain. (See, e.g., Bird *et al.*, *Science* 242:423-426 (1988) and Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988).) In some cases, the antibodies are diabodies, *i.e.*, are
15 bivalent antibodies in which V_H and V_L domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites. (See, e.g., Holliger P. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:6444-6448 (1993), and Poljak R. J. *et al.*, *Structure* 2:1121-1123 (1994).) In some cases, one or more CDRs from an antibody herein may be incorporated into a molecule either covalently or noncovalently to make it an immunoadhesin that specifically binds to CCR2. In such cases, the CDR(s) may be incorporated as part of a larger
20 polypeptide chain, may be covalently linked to another polypeptide chain, or may be incorporated noncovalently. In cases having one or more binding sites, the binding sites may be identical to one another or may be different.

[0043] As used herein, the term "human antibody" means any antibody in which the variable and constant domain sequences are human sequences. The term
30 encompasses antibodies with sequences derived from human genes, but which have been changed, e.g., to decrease possible immunogenicity, increase affinity, eliminate cysteines that might cause undesirable folding, etc. The term encompasses such antibodies produced recombinantly in non-human cells, which

might impart glycosylation not typical of human cells. These antibodies may be prepared in a variety of ways, as described herein.

5 [0044] The term "chimeric antibody" as used herein means an antibody that comprises regions from two or more different antibodies. In one case, one or more of the CDRs of the chimeric antibody are derived from a human anti-CCR2 antibody. In another case, all of the CDRs are derived from human anti-CCR2 antibodies. In another case, the CDRs from more than one human anti-CCR2 antibodies are combined in a chimeric antibody. For instance, a chimeric antibody may comprise a CDR1 from the light chain of a first human anti-CCR2 antibody, a
10 CDR2 from the light chain of a second human anti-CCR2 antibody and a CDR3 from the light chain of a third human anti-CCR2 antibody, and CDRs from the heavy chain may be derived from one or more other anti-CCR2 antibodies. Further, the framework regions may be derived from one of the anti-CCR2 antibodies from which one or more of the CDRs are taken or from one or more
15 different human antibodies.

[0045] In some cases, a chimeric antibody is a humanized anti-CCR2 antibody. A humanized anti-CCR2 antibody comprises the amino acid sequence of one or more framework regions and/or the amino acid sequence from at least a portion of the constant region of one or more human anti-CCR2 antibodies and CDRs
20 derived from a non-human anti-CCR2 antibody.

[0046] Fragments or analogs of antibodies or immunoglobulin molecules can be readily prepared by those of ordinary skill in the art following the teachings of this specification. Preferred amino- and carboxy-termini of fragments or analogs occur near boundaries of functional domains.

25 [0047] The term "surface plasmon resonance," as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.). For further descriptions, see Jonsson U. *et al.*,
30 *Ann. Biol. Clin.* 51:19-26 (1993); Jonsson U. *et al.*, *Biotechniques* 11:620-627 (1991); Jonsson B. *et al.*, *J. Mol. Recognit.* 8:125-131 (1995); and Johnsson B. *et al.*, *Anal. Biochem.* 198:268-277 (1991).

[0048] The term "K_D" refers to the equilibrium dissociation constant of a particular antibody-antigen interaction. An antibody is said to specifically bind an antigen when the dissociation constant is ≤ 1 mM, ≤ 100 nM, or ≤ 10 nM. In certain cases, the K_D is 1 pM to 500 pM. In other cases, the K_D is between 500 pM to 1 μ M, 1 μ M to 100 nM, or 100 nM to 10 nM.

[0049] The term "epitope" includes any protein determinant capable of specific binding to an immunoglobulin or T-cell receptor or otherwise interacting with a molecule. Epitopic determinants generally consist of chemically-active surface groupings of molecules such as amino acids or carbohydrate or sugar side chains and generally have specific three dimensional structural characteristics, as well as specific charge characteristics. An epitope may be "linear" or "conformational." In a linear epitope, all of the points of interaction between the protein and the interacting molecule (such as an antibody) occur linearly along the primary amino acid sequence of the protein. In a conformational epitope, the points of interaction occur across amino acid residues on the protein that are separated from one another. Once a desired epitope on an antigen is determined, it is possible to generate antibodies to that epitope, e.g., using the techniques described in the present specification. Alternatively, during the discovery process, the generation and characterization of antibodies may elucidate information about desirable epitopes. From this information, it is then possible to competitively screen antibodies for binding to the same epitope. An approach to achieve this is to conduct competition and cross-competition studies to find antibodies that compete or cross-compete with one another for binding to CCR2, e.g., the antibodies compete for binding to the antigen. A high throughput process for "binning" antibodies based upon their cross-competition is described in International Patent Application No. WO 03/48731.

[0050] As used herein, the twenty conventional amino acids and their abbreviations follow conventional usage. See Immunology - A Synthesis (second Edition, E.S. Golub and D.R. Gren, Eds., Sinauer Associates, Sunderland, Mass. (1991)), incorporated herein by reference.

[0051] The term "polynucleotide" as referred to herein means a polymeric form of nucleotides of at least 10 bases in length, either ribonucleotides or

deoxyribonucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms.

[0052] The term "isolated polynucleotide" as used herein means a polynucleotide of genomic, cDNA, or synthetic origin or some combination thereof, which by virtue of its origin the "isolated polynucleotide" (1) is not associated with all or a portion of a polynucleotides with which the "isolated polynucleotide" is found in nature, (2) is operably linked to a polynucleotide to which it is not linked in nature, or (3) does not occur in nature as part of a larger sequence.

[0053] The term "naturally occurring nucleotides" as used herein includes deoxyribonucleotides and ribonucleotides. The term "modified nucleotides" as used herein includes nucleotides with modified or substituted sugar groups and the like. The term "oligonucleotide linkages" referred to herein includes oligonucleotides linkages such as phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate, phosphoroanilothioate, phosphoraniladate, phosphoroamidate, and the like. See e.g., LaPlanche *et al.*, *Nucl. Acids Res.* 14:9081 (1986); Stec *et al.*, *J. Am. Chem. Soc.* 106:6077 (1984); Stein *et al.*, *Nucl. Acids Res.* 16:3209 (1988); Zon *et al.*, *Anti-Cancer Drug Design* 6:539 (1991); Zon *et al.*, Oligonucleotides and Analogues: A Practical Approach, pp. 87-108 (F. Eckstein, Ed., Oxford University Press, Oxford England (1991)); U.S. Patent No. 5,151,510; Uhlmann and Peyman, *Chemical Reviews* 90:543 (1990), the disclosures of which are hereby incorporated by reference. An oligonucleotide can include a label for detection, if desired.

[0054] "Operably linked" sequences include both expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. The term "expression control sequence" as used herein means polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (*i.e.*, Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such

control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term
5 "control sequences" is intended to include, at a minimum, all components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

[0055] The term "vector", as used herein, means a nucleic acid molecule capable
10 of transporting another nucleic acid to which it has been linked. In some cases, the vector is a plasmid, *i.e.*, a circular double stranded piece of DNA into which additional DNA segments may be ligated. In some cases, the vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. In some cases, the vectors are capable of autonomous replication in a host cell into
15 which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). In other cases, the vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression
20 of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors").

[0056] The term "recombinant host cell" (or simply "host cell"), as used herein, means a cell into which a recombinant expression vector has been introduced. It should be understood that "recombinant host cell" and "host cell" mean not only
25 the particular subject cell but also the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

[0057] The term "percent sequence identity" in the context of nucleotide
30 sequences means the residues in two sequences that are the same when aligned for maximum correspondence. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using FASTA, Gap or

Bestfit, which are programs in Wisconsin Package Version 10.0, Genetics Computer Group (GCG), Madison, Wisconsin. FASTA, which includes, e.g., the programs FASTA2 and FASTA3, provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000); Pearson, *Methods Enzymol.* 266:227-258 (1996); Pearson, *J. Mol. Biol.* 276:71-84 (1998); incorporated herein by reference).

Unless otherwise specified, default parameters for a particular program or algorithm are used. For instance, percent sequence identity between nucleotide sequences can be determined using FASTA with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) or using Gap with its default parameters as provided in GCG Version 6.1, incorporated herein by reference.

[0058] A reference to a nucleotide sequence encompasses its complement unless otherwise specified. Thus, a reference to a nucleic acid having a particular sequence should be understood to encompass its complementary strand, with its complementary sequence.

[0059] As used herein, the terms "percent sequence identity" and "percent sequence homology" are used interchangeably.

[0060] The term "substantial similarity" or "substantial sequence similarity," when referring to a nucleic acid or fragment thereof, means that when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 85%, at least about 90%, and at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or Gap, as discussed above.

[0061] As applied to polypeptides, the term "substantial identity" means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights as supplied with the programs, share at least 70%, 75% or 80% sequence identity, at least 90% or 95% sequence identity, and at least 97%, 98% or 99% sequence identity. In certain cases, residue positions that are not identical differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is

substituted by another amino acid residue having a side chain R group with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. See, e.g., Pearson, *Methods Mol. Biol.* 243:307-31 (1994). Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine, and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartic acid and glutamic acid; and 7) sulfur-containing side chains: cysteine and methionine. Conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine.

[0062] Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.*, *Science* 256:1443-45 (1992), incorporated herein by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[0063] Sequence identity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters as specified by the programs to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, e.g., GCG Version 6.1 (University of Wisconsin, WI). Polypeptide sequences also can be compared using FASTA using default or recommended parameters, see GCG Version 6.1. FASTA (e.g., FASTA2 and FASTA3) provides

alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, *Methods Enzymol.* 183:63-98 (1990); Pearson, *Methods Mol. Biol.* 132:185-219 (2000)). Another preferred algorithm when comparing a sequence to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn, using default parameters as supplied with the programs. See, e.g., Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990); Altschul *et al.*, *Nucleic Acids Res.* 25:3389-402 (1997).

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[0064] CCR2 is a seven-transmembrane domain protein, and accordingly it has six loops. Loops 1, 3 and 5, counting from the extracellular N-terminus, are intracellular loops, while loops 2, 4 and 6 are extracellular. The first, second and third extracellular loops of CCR2 refer to loops 2, 4 and 6, respectively.

[0065] Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

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Human Anti-CCR2 Antibodies and Characterization Thereof

[0066] In some cases, human anti-CCR2 antibodies are provided. In some cases, human anti-CCR2 antibodies are produced by immunizing a non-human transgenic animal, e.g., a rodent, whose genome comprises human immunoglobulin genes so that the transgenic animal produces human antibodies. In some cases, the anti-CCR2 antibodies and antigen-binding portions include, but are not limited to, antibodies or antigen-binding portions (i) which bind to the first or second extracellular loop of CCR2, or both; (ii) which do not bind to the N-terminal end or the third extracellular loop of CCR2 or both; or (iii) which do both (i) and (ii). In another case, human anti-CCR2 antibodies that bind to a polypeptide comprising the amino acid sequence of SEQ ID NO:128 or SEQ ID NO:129 are provided. In another case, human anti-CCR2 antibodies bind to a polypeptide comprising an amino acid sequence 80, 85, 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:128 or SEQ ID NO:129. In another case, the anti-CCR2 antibodies and antigen-binding portions include, but are not limited to,

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antibodies or antigen-binding portions which bind to the third extracellular loop of CCR2.

[0067] The V_H , V_K , V_λ genes are classified into families on the basis of sequence homology. Two V_H , V_K , or V_λ genes belong to the same family if they share the same nucleotide sequence at more than 80% of the positions. An anti-CCR2 antibody may comprise a human kappa light chain (V_K) or a human-lambda light chain (V_λ) or an amino acid sequence derived there from. In some cases comprising a lambda light chain, the light chain variable domain (V_L) utilizes a human $V_{\lambda 1}$, $V_{\lambda 2}$, $V_{\lambda 3}$, $V_{\lambda 4}$, $V_{\lambda 5}$, $V_{\lambda 6}$, $V_{\lambda 7}$, $V_{\lambda 8}$, $V_{\lambda 9}$, or $V_{\lambda 10}$ family gene (Williams S.C. *et al. J. Mol. Bio.* 264:220-232 (1996)).

[0068] In some cases comprising a kappa light chain, the light chain variable domain (V_L) utilizes a human $V_{K I}$, $V_{K II}$, $V_{K III}$, $V_{K IV}$, $V_{K V}$, or $V_{K VI}$ family gene (Cox J. P. L., *et al., Eur. J. Immunol* 24:827-836 (1994)), preferably a $V_{K I}$, $V_{K II}$, $V_{K IV}$, or $V_{K VI}$ family gene, preferably a $V_{K I}$ or $V_{K VI}$ family gene. In some cases, the light chain germline sequence is selected from human V_K sequences including, but not limited to, A1, A10, A11, A14, A17, A18, A19, A2, A20, A23, A26, A27, A3, A30, A5, A7, B2, B3, L1, L10, L11, L12, L14, L15, L16, L18, L19, L2, L20, L22, L23, L24, L25, L4/18a, L5, L6, L8, L9, O1, O11, O12, O14, O18, O2, O4, and O8. In certain cases, this light chain human germline gene is selected from V1-11, V1-13, V1-16, V1-17, V1-18, V1-19, V1-2, V1-20, V1-22, V1-3, V1-4, V1-5, V1-7, V1-9, V2-1, V2-11, V2-13, V2-14, V2-15, V2-17, V2-19, V2-6, V2-7, V2-8, V3-2, V3-3, V3-4, V4-1, V4-2, V4-3, V4-4, V4-6, V5-1, V5-2, V5-4, and V5-6. In certain cases, the light chain utilizes a human $V_{K I}$ O12, $V_{K II}$ A1, a $V_{K IV}$ B3 or a $V_{K VI}$ A26 germline gene.

[0069] An anti-CCR2 antibody may comprise a heavy chain variable domain (V_H) that utilizes a human $V_{H 1}$, $V_{H 2}$, $V_{H 3}$, $V_{H 4}$, $V_{H 5}$, $V_{H 6}$ or $V_{H 7}$ family gene. In particular examples, this heavy chain human germline gene is selected from VH1-18, VH1-2, VH1-24, VH1-3, VH1-45, VH1-46, VH1-58, VH1-69, VH1-8, VH2-26, VH2-5, VH2-70, VH3-11, VH3-13, VH3-15, VH3-16, VH3-20, VH3-21, VH3-23, VH3-30, VH3-33, VH3-35, VH3-38, VH3-43, VH3-48, VH3-49, VH3-53, VH3-64, VH3-66, VH3-7, VH3-72, VH3-73, VH3-74, VH3-9, VH4-28, VH4-31, VH4-34, VH4-39, VH4-4, VH4-59, VH4-61, VH5-51, VH6-1, and VH7-81. In certain cases, the heavy chain utilizes a human $V_{H I}$ 1-46 or a $V_{H III}$ 3-30 gene.

[0070] In particular cases, the light chain variable region and/or heavy chain variable region comprises a framework region or at least a portion of a framework region (e.g., containing 2 or 3 subregions, such as FR2 and FR3). In certain cases, at least FRL1, FRL2, FRL3, or FRL4 is fully human. In other examples, at least FRH1, FRH2, FRH3, or FRH4 is fully human. In some cases, at least FRL1, FRL2, FRL3, or FRL4 is a germline sequence (e.g., human germline) or comprises human consensus sequences for the particular framework (readily available at the sources of known human Ig sequences described herein). In other examples, at least FRH1, FRH2, FRH3, or FRH4 is a germline sequence (e.g., human germline) or comprises human consensus sequences for the particular framework.

[0071] In some cases, the V_L of the CCR2 antibody comprises one or more amino acid substitutions, deletions, and/or insertions relative to the germline amino acid sequence of the human gene. In some cases, the V_L of the anti-CCR2 antibody comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions relative to the germline amino acid sequence. In some cases, one or more of those substitutions, deletions, and/or insertions is in a CDR of the light chain. In some cases, the amino acid substitutions, deletions and/or insertions relative to germline are at one or more of the same positions as the substitution, deletion and/or insertion relative to germline in any one or more of the V_L of antibodies 4.40, 4.9, 4.22, 4.39 or 4.40 A68G S230P. For example, the V_L of an anti-CCR2 antibody may contain one or more amino acid substitutions, deletions, and/or insertions compared to germline found in the V_L of antibody 4.40. In some cases, the amino acid changes are at one or more of the same positions, but involve a different substitution, deletion and/or insertion compared to germline. In some cases, substitution may represent conservative amino acid substitutions at such position(s) relative to the amino acid in the reference antibody. For example, if a particular position in one of these antibodies is substituted relative to germline and is glutamate, one may substitute aspartate at that position. Similarly, if an amino acid substitution compared to germline is serine, one may conservatively substitute threonine for serine at that position.

[0072] In some cases, the light chain of the human anti-CCR2 antibody comprises the variable domain (V_L) amino acid sequence of antibody 4.40 (SEQ ID NO:101);

4.9 (SEQ ID NO:29); 4.22 (SEQ ID NO:65); 4.39 (SEQ ID NO:194); or 4.40 A68G S230P (SEQ ID NO:113); or said amino acid sequence having up to 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions and/or a total of up to 3 non-conservative amino acid substitutions. In some cases, the light chain may comprise CDR1, CDR2 and CDR3 independently selected from the light chain CDR1, CDR2 and CDR3, respectively of the light chain of antibody 4.40, 4.9, 4.22, 4.39 or 4.40 A68G S230P, or CDRs each having less than 4 or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions. In some cases, the light chain of the anti-CCR2 antibody comprises a light chain CDR1, CDR2, and CDR3, each of which is independently selected from the light chain CDR1, CDR2 and CDR3 regions of monoclonal antibody 4.40 (SEQ ID NO:100); 4.9 (SEQ ID NO:28); 4.22 (SEQ ID NO:64); 4.39 (SEQ ID NO:193); or 4.40 A68G S230P (SEQ ID NO:112). In certain cases, the light chain of the anti-CCR2 antibody comprises the light chain CDR1, CDR2 and CDR3 of an antibody comprising the amino acid sequence of the V_L region of an antibody selected from 4.40 (SEQ ID NO:101); 4.9 (SEQ ID NO:29); 4.22 (SEQ ID NO:65); 4.39 (SEQ ID NO:194); or 4.40 A68G S230P (SEQ ID NO:113); or said CDRs each having less than 4 or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions. Sequence identifiers are listed for the CDRs of certain antibodies in Table 8.

[0073] With regard to the heavy chain, in some cases, the variable domain (V_H) utilizes a human V_H 3-30, or V_H 1-46 gene sequence. In some cases, the V_H sequence of the anti-CCR2 antibody contains one or more amino acid substitutions, deletions and/or insertions (additions) relative to the germline amino acid sequence. In some cases, the variable domain of the heavy chain comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 substitutions, deletions and/or insertions from the germline amino acid sequence. In some cases, the substitution is a non-conservative substitution compared to the germline amino acid sequence. In some cases, the substitution, deletion and/or insertion are in a CDR of the heavy chain. In some cases, the amino acid substitution, deletion and/or insertion are made at one or more of the same positions as the mutations from germline in any one or more of the V_H of antibodies 4.40, 4.22, 4.39 or 4.9.

In other cases, the amino acid substitution, deletion and/or insertion are at one or more of the same positions but involve a different substitution, deletion and/or insertion than in the reference antibody. In some cases the antibody comprises a heavy chain CDR3 having the amino acid sequence of SEQ ID NO:202 or SEQ ID NO:203.

[0074] In some cases, the heavy chain comprises the V_H amino acid sequence of antibody 4.40 (SEQ ID NO:83); 4.22 (SEQ ID NO:47); 4.9 (SEQ ID NO:11); 4.39 (SEQ ID NO:176); or said V_H amino acid sequence having up to 1, 2, 3, 4, 6, 8, or 10 conservative amino acid substitutions and/or a total of up to 3 non-conservative amino acid substitutions. In some cases, the heavy chain comprises the amino acid sequence from the beginning of the CDR1 to the end of the CDR3 of any one of the foregoing antibodies.

[0075] In some cases, the heavy chain comprises the heavy chain CDR1, CDR2 and CDR3 of antibody 4.40, 4.22, 4.39 or 4.9 or said CDRs each having less than 8, less than 6, less than 4, or less than 3 conservative amino acid substitutions and/or a total of three or fewer non-conservative amino acid substitutions.

[0076] In some cases, the heavy chain CDRs are independently selected from the CDRs of antibodies 4.40, 4.22, 4.39 or 4.9. In another case, the heavy chain comprises CDRs independently selected from two or more V_H regions selected from 4.40 (SEQ ID NO:83); 4.22 (SEQ ID NO:47); 4.39 (SEQ ID NO:176) or 4.9 (SEQ ID NO:11). In another case, the antibody comprises a light chain as disclosed above and a heavy chain as disclosed above. In a further case, the light chain CDRs and the heavy chain CDRs are from the same antibody.

[0077] One type of amino acid substitution that may be made is to change one or more cysteines in the antibody, which may be chemically reactive, to another residue, such as, without limitation, alanine or serine. In one case, there is a substitution of a non-canonical cysteine. The substitution can be made in a CDR or framework region of a variable domain or in the constant domain of an antibody. In some cases, the cysteine is canonical.

[0078] Another type of amino acid substitution that may be made is to change any potential proteolytic sites in the antibody. Such sites may occur in a CDR or framework region of a variable domain or in the constant domain of an antibody. Substitution of cysteine residues and removal of proteolytic sites may decrease

the risk of any heterogeneity in the antibody product and thus increase its homogeneity. Another type of amino acid substitution is to eliminate asparagine-glycine pairs, which form potential deamidation sites, by altering one or both of the residues. In some cases, amino acid substitution are used to insert or remove a glycosylation site. In some cases, the C-terminal lysine of the heavy chain of the anti-CCR2 antibody may be proteolytically or genetically removed. In various cases, the heavy and light chains of the anti-CCR2 antibodies may optionally include a signal sequence.

[0079] In one aspect, the antibodies are produced by a hybridoma.

[0080] Table 1 lists the sequence identifiers (SEQ ID NOs) of the nucleic acids encoding the full-length, and variable domain-comprising portions, of heavy and light chains, and the corresponding deduced amino acid sequences of exemplary antibodies.

TABLE 1

HUMAN ANTI-CCR2 ANTIBODIES								
Monoclonal Antibody	SEQUENCE IDENTIFIER (SEQ ID NO:)							
	Variable Domain Comprising Portion				Full Length			
	Heavy (V _H)		Light (V _L)		Heavy		Light	
	Protein	DNA	Protein	DNA	Protein	DNA	Protein	DNA
4.40.2	83	74	101	92	82	73	100	91
4.40.2 A68G S230P	83	74	113	110	116	115	112	109
4.9.2	11	2	29	20	10	1	28	19
4.22.3	47	38	65	56	46	37	64	55
4.39.3	176	167	194	185	175	166	193	184

[0081] Also provided are heavy and/or light chain variants of certain of the above-listed human anti-CCR2 antibodies, comprising one or more amino acid substitutions. To designate the variants, the first letter is the one letter symbol for the amino acid of the naturally-occurring antibody chain, the number refers to the

position of the amino acid (wherein position one is the N-terminal amino acid), and the second letter is the one letter symbol for the variant amino acid. In some cases, heavy chain variants are provided. One such heavy chain variant is a hinge region stabilizing mutation to reduce formation of half-monomer (Angal, S. et al. *Molecular Immunology* 30:105-108 (1993)). One such hinge stabilizing mutation of the 4.40 antibody heavy chain variant has a proline substitution for serine at position 230 of SEQ ID NO:82. The DNA sequence encoding the S230P variant has a CCA codon beginning at position 688 of SEQ ID NO:115.

[0082] Also provided are variant light chains of monoclonal antibody 4.40. A68G is a 4.40 light chain variant, represented by SEQ ID NO:113, in which residue 68 is a glycine residue. In the DNA sequence, the A68G 4.40 variant is encoded by SEQ ID NO:109, in which the codon beginning at position 252 is GGG.

[0083] In other cases, antibodies containing combinations of amino acid variants can be produced. An example of a combination of variants is the anti-CCR2 antibody 4.40 A68G S230P, which comprises the light chain substitution A68G and the heavy chain substitution S230P in the context of the 4.40 antibody. Further combinations of a variant heavy chain and a variant light chain of 4.40 are included.

[0084] In one case, the anti-CCR2 antibody is 4.40, 4.22, 4.39, 4.40 A68G S230P or 4.9. In still further cases, included are antibodies comprising variable domain amino acid sequences with more than 80%, more than 85%, more than 90%, more than 95%, more than 96%, more than 97%, more than 98% or more than 99% sequence identity to an variable domain amino acid sequence of any of the above-listed human anti-CCR2 antibodies.

25 Class and Subclass of Anti-CCR2 Antibodies

[0085] The class and subclass of anti-CCR2 antibodies may be determined by any method known in the art. In general, the class and subclass of an antibody may be determined using antibodies that are specific for a particular class and subclass of antibody. Such antibodies are commercially available. The class and subclass can be determined by ELISA, or Western Blot as well as other techniques. Alternatively, the class and subclass may be determined by sequencing all or a portion of the constant domains of the heavy and/or light

chains of the antibodies, comparing their amino acid sequences to the known amino acid sequences of various class and subclasses of immunoglobulins, and determining the class and subclass of the antibodies.

[0086] In some cases, the anti-CCR2 antibody is a monoclonal antibody. The anti-CCR2 antibody can be an IgG, an IgM, an IgE, an IgA, or an IgD molecule. In one case, the anti-CCR2 antibody is an IgG, belonging to, e.g., an IgG1, IgG2, IgG3, or IgG4 subclass. In another case, the anti-CCR2 antibody is an IgG4. In still another case, the antibody is an IgG4 isoallotype (Ellison J. and Hood L., *PNAS* 79:1984-1988 (1982); & Brusco A. *et al.*, *Eur J. Immunogenetics* 25:349-355 (1998)).

Binding Affinity of Anti-CCR2 Antibodies to CCR2

[0087] In some cases, the anti-CCR2 antibodies bind to CCR2 with high affinity.

[0088] In some cases, the anti-CCR2 antibodies bind with high affinity to the first extracellular loop of CCR2, to the second extracellular loop of CCR2, or to an epitope formed by both the 1st and 2nd extracellular loops.

[0089] In a related case, the anti-CCR2 antibodies bind to a polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:128 or in SEQ ID NO:129.

[0090] In some cases, the anti-CCR2 antibodies do not bind to the third extracellular loop of CCR2 or to the N-terminal domain of CCR2.

[0091] In another case, the anti-CCR2 antibodies do not bind to a peptide consisting of the sequence set forth in SEQ ID NO:127 or in SEQ ID NO:130. In another case, the anti-CCR2 antibody binds to the first and/or second extracellular loops of CCR2, with a K_D of about 2×10^{-7} M or less, with a K_D of about 2×10^{-8} M or less, with a K_D of about 2×10^{-9} M or less, with a K_D of about 1×10^{-9} M or less, with a K_D of about 9×10^{-10} M or less, with a K_D of about 8×10^{-10} M or less, with a K_D of about 7×10^{-10} M or less, with a K_D of about 6×10^{-10} M or less, with a K_D of about 5×10^{-10} M or less, with a K_D of about 4×10^{-10} M or less, with a K_D of about 3×10^{-10} M or less, or with a K_D of about 2×10^{-10} M or less. In certain cases, the antibody binds to CCR2, or to the first and/or second extracellular loops of CCR2, with substantially the same K_D as an antibody selected from 4.40, 4.9, 4.22, 4.39 or 4.40 A68G S230P. In still another case, the antibody binds to CCR2, or to the

first and/or second extracellular loops of CCR2, with substantially the same K_D as an antibody that comprises a heavy chain variable domain having the amino acid sequence of the V_H region found in SEQ ID NO:83, SEQ ID NO:11, SEQ ID NO:176 or SEQ ID NO:47. In still another case, the antibody binds to CCR2 with

5 substantially the same K_D as an antibody that comprises the CDRs of a light chain variable domain having the amino acid sequence of the V_L region found in SEQ ID NO:101, SEQ ID NO:113, SEQ ID NO:29, SEQ ID NO:194 or SEQ ID NO:65, or that comprises the CDRs of a heavy chain variable domain having the amino acid sequence the V_H region found in SEQ ID NO:83, SEQ ID NO:11, SEQ

10 ID NO:176 or SEQ ID NO:47.

[0092] In some cases, the anti-CCR2 antibody may have a low dissociation rate constant (k_{off}). In some cases, the anti-CCR2 antibody may bind to CCR2, or more preferably to the first and/or second extracellular loops of CCR2, with a k_{off} of $1.0 \times 10^{-3} \text{ s}^{-1}$ or lower, a k_{off} of $5.0 \times 10^{-4} \text{ s}^{-1}$ or lower or a k_{off} of $2 \times 10^{-4} \text{ s}^{-1}$ or

15 lower. In some cases, the k_{off} may be substantially the same as an antibody described herein, including an antibody selected from 4.40, 4.9, 4.22, 4.39 and 4.40 A68G S230P. In some cases, the antibody may bind to CCR2, or to the first and/or second extracellular loops of CCR2, with substantially the same k_{off} as an antibody that comprises the CDRs of a heavy chain, or the CDRs of a light chain,

20 from an antibody selected from 4.40, 4.9 and 4.40 A68G S230P. In some cases, the antibody may bind to CCR2, or to the first and/or second extracellular loops of CCR2, with substantially the same k_{off} as an antibody that comprises (i) a heavy chain variable domain having the amino acid sequence of the V_H region found in SEQ ID NO:83, or SEQ ID NO:11, (ii) a light chain variable domain having the amino acid sequence of the V_L region found in SEQ ID NO:101, SEQ ID NO:113,

25 SEQ ID NO:29, or (iii) both (i) and (ii). In still another case, the antibody may bind to CCR2, or to the first and/or second extracellular loops of CCR2, with substantially the same k_{off} as an antibody that comprises the CDRs of a light chain variable domain having the amino acid sequence of the V_L region found in SEQ ID

30 NO:101, SEQ ID NO:113, or SEQ ID NO:29; and the CDRs of a heavy chain variable domain having the amino acid sequence of the V_H region found in SEQ ID NO:83 or SEQ ID NO:11.

[0093] The binding affinity and dissociation rate of an anti-CCR2 antibody to CCR2 can be determined by methods known in the art. The binding affinity can be measured by ELISAs, RIAs, flow cytometry, surface plasmon resonance, such as BIACORE™. The dissociation rate can be measured by surface plasmon resonance. One can determine whether an antibody has substantially the same K_D as an anti-CCR2 antibody by using methods known in the art. Example 5 exemplifies a method for determining affinity constants of anti-CCR2 monoclonal antibodies.

Identification of CCR2 Epitopes Recognized by Anti-CCR2 Antibodies

10 [0094] Provided are human anti-CCR2 monoclonal antibodies that binds to CCR2 and may compete or cross-compete with and/or binds the same epitope as: (a) an antibody selected from 4.40, 4.9, 4.22, 4.39 and 4.40 A68G S230P; (b) an antibody that comprises a heavy chain variable domain having an amino acid sequence of the variable domain found in SEQ ID NO:83, SEQ ID NO:11, SEQ ID NO:176 or SEQ ID NO:47, (c) an antibody that comprises a light chain variable domain having an amino acid sequence of the variable domain found in SEQ ID NO:101, SEQ ID NO:113, SEQ ID NO:29, SEQ ID NO:194, or SEQ ID NO:65, or (d) an antibody that comprises both a heavy chain variable domain as defined in (b) and a light chain variable domain as defined in (c). If two antibodies reciprocally compete with each other for binding to CCR2, they are said to cross-compete.

[0095] One can determine whether an antibody binds to the same epitope, competes or cross-competes for binding with an anti-CCR2 antibody provided herein by using methods known in the art. In one case, one allows the anti-CCR2 antibody provided herein to bind to CCR2 under saturating conditions and then measures the ability of the test antibody to bind to CCR2. If the test antibody is able to bind to CCR2 at the same time as the provided anti-CCR2 antibody, then the test antibody binds to a different epitope as the anti-CCR2 antibody. However, if the test antibody is not able to bind to CCR2 at the same time, then the test antibody binds to the same epitope, an overlapping epitope, or an epitope that is in close proximity to the epitope bound by the human anti-CCR2 antibody provided herein. To determine if a test antibody cross-competes with a reference

antibody, the experiment is conducted reversing the antibodies, i.e., one allows the test antibody to bind to CCR2 and then measures the ability of the anti-CCR2 antibody provided herein to bind CCR2. These experiments can be performed using ELISA, RIA, BIACORE™, or flow cytometry (FACS).

5 Inhibition of CCR2 Activity by Anti-CCR2 Antibody

[0096] In some cases, provided are anti-CCR2 antibodies that inhibit CCR2-mediated signaling. In other cases, provided are anti-CCR2 antibodies that inhibit MCP-1, MCP-2, MCP-3, and/or MCP-4 mediated signaling through CCR2. In other cases, provided are anti-CCR2 antibodies that inhibit the binding of MCP-1
10 MCP-2, MCP-3 and/or MCP-4 to CCR2. In one case, the CCR2 is human CCR2. In some cases, the CCR2 is human CCR2A, CCR2B or both. In still another case, the anti-CCR2 antibody is a human antibody.

[0097] The IC₅₀ of an anti-CCR2 antibody can be measured in ligand binding assays such as ELISA, RIA, or related assays and cell-based assays such as
15 chemotaxis assays of cells expressing CCR2. In various cases, the antibody or an antigen-binding portion thereof inhibits ligand binding between MCP-1 and CCR2 with an IC₅₀ of no more than 5 µg/ml, no more than 1 µg/ml, no more than 0.5 µg/ml, or no more than 0.20 µg/ml as measured by an ELISA assay.

[0098] In another case, an anti-CCR2 antibody that reduces activation of CCR2 in
20 the presence of CCR2 ligands such as MCP-1 (CCL2), MCP-2, MCP-3, and/or MCP-4 is provided. In one case, the anti-CCR2 antibody may inhibit CCR2 ligand induced (i) G-protein activation, (ii) adenylate cyclase activation, (iii) mitogen-activated protein kinases (MAPKs) activation, (iv) cytosolic calcium mobilization, (v) ERK phosphorylation, (vi) chemotaxis, or (vii) actin polymerization. One can
25 determine whether an anti-CCR2 antibody can prevent, inhibit or reduce activation of CCR2 in the presence of MCP-1 by determining the GTP/GDP ratio of G-proteins in cells labeled with radiolabeled GTP, by measuring GTPγS incorporation, by measuring cytosolic calcium influx using calcium chromophores, or by measuring the phosphorylation state of MAPKs in a cell. Assays for
30 detecting CCR2 activation and/or MCP-1 binding to CCR2 are described, for example, in Gabrilin *et al.*, *Biochem Biophys Res Commun.* 327(2):533-40 (2005), and Jimenez-Sainz *et al.*, *Mol Pharmacol.* 64(3):773-82 (2003).

[0099] In one case, one would determine the levels of CCR2 activation using a chemotaxis assay. In some cases, the IC_{50} , measured using a chemotaxis assay, is no more than 5 $\mu\text{g/ml}$, no more than 1 $\mu\text{g/ml}$, no more than 0.5 $\mu\text{g/ml}$, or no more than 0.20 $\mu\text{g/ml}$. Example 10 exemplifies one type of assay that measures inhibition of CCR2 by an anti-CCR2 antibody by monitoring calcium mobilization.

[00100] In another aspect, contacting a cell with the antibody may result in a down regulation of cell surface CCR2 expression after incubation with the antibody. In some cases, the incubation can be a short time period (e.g., 4 hours) or a longer time period (e.g., 24 hours). A down regulation of cell surface CCR2 expression can be measured using Western blotting, ELISA, or FACS analysis. In particular cases, contacting a cell with the antibody may result in at least a 6% decrease, at least a 10% decrease, at least a 20% decrease, at least a 30% decrease, or at least a 50% decrease of cell surface CCR2 expression as measured by Western blotting or ELISA.

[00101] In another aspect, the antibody reduces MCP-1 induced pERK phosphorylation. A down regulation of MCP-1 induced pERK phosphorylation can be measured using Western blotting, ELISA, or FACS analysis. In particular cases, the antibody produces at least a 6% decrease, at least a 10% decrease, at least a 20% decrease, at least a 30% decrease, or at least a 50% decrease of MCP-1 induced pERK phosphorylation as measured by FACS analysis.

Inhibition of Chemotaxis *In Vivo* with Anti-CCR2 Antibodies

[00102] According to some cases, provided are anti-CCR2 antibodies that inhibit the chemotaxis of immune cells *in vivo*. Immune cells whose chemotaxis is inhibited include peripheral blood mononuclear cells, THP cells, monocytes, memory T lymphocytes, dendritic cells, basophils, natural killer cells and adoptively transferred CCR2+ cells. In one case, the anti-CCR2 antibody inhibits immune cell chemotaxis in response to one or more of MCP-1, MCP-2, MCP-3 and MCP-4. In one case, the chemokine is MCP-1. In still another case, the chemokine is MCP-3. The anti-CCR2 antibody may inhibit chemotaxis to sites of inflammation or injury.

[00103] According to some cases, also provided are anti-CCR2 antibodies that inhibit the chemotaxis of non-immune cells, including but not limited to, fibroblast-

like synoviocytes (FLS) (see Garcia-Vicuna *et al.*, *Arthritis Rheum.* 50(12):3866-77 (2004)), adult neural stem cells (see Widera *et al.*, *Eur J Cell Biol.* 83(8):381-7 (2004)) and human fetal astrocytes (see Andjelkovic *et al.*, *J Neurosci Res.* 70(2):219-31 (2002)).

- 5 **[00104]** In one case, the antibody inhibits cell chemotaxis as compared to the chemotaxis of cells in an untreated animal. In another case, the anti-CCR2 antibody reduces chemotaxis by at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%. In one case, the inhibition of chemotaxis is measured at least 1 hour after the animals have started treatment
10 with the antibody. In another case, the inhibition of chemotaxis is measured at least 7 days after the animals have started treatment with the antibody. In another case, the anti-CCR2 antibody results in chemotaxis inhibition of at least 10% to 100%.

Species and Molecular Selectivity

- 15 **[00105]** In another aspect, the anti-CCR2 antibodies demonstrate both species and molecular selectivity. In some cases, the anti-CCR2 antibody binds to human and cynomolgus CCR2. The anti-CCR2 antibody may bind to additional CCR2 of non-human primate species. In some cases, the anti-CCR2 antibody does not bind to mouse or rat CCR2. Following the teachings of the specification, one may
20 determine the species selectivity for the anti-CCR2 antibody using methods well known in the art. For instance, one may determine the species selectivity using Western blot, flow cytometry, ELISA, immunoprecipitation or RIA. In one case, one may determine the species selectivity using flow cytometry. In another case, one may determine species specificity by assessing the ability of the antibody to
25 inhibit MCP-1 functional responses using cells from that species. This may include chemotaxis, actin polymerization, calcium mobilization, etc.

- [00106]** In another case, the anti-CCR2 antibody has a selectivity for the polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:126 (human CCR2B) over the polypeptide consisting of the amino acid sequence set
30 forth in SEQ ID NO:131 (human CCR5). In another case, the anti-CCR2 antibody has selectivity for CCR2 over CCR5 of at least 2-fold, at least 5 fold, at least 10 fold, at least 25 fold, at least 50 fold, or at least 100 fold. In another case, the

human anti-CCR2 antibodies bind to a polypeptide comprising an amino acid sequence 80, 85, 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:126.

[00107] In another case, the anti-CCR2 antibody may have a selectivity for the polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:125

5 (human CCR2A) over the polypeptide consisting of the amino acid sequence set forth in SEQ ID NO:131 (human CCR5) of at least 2-fold, at least 5 fold, at least 10 fold, at least 25 fold, at least 50 fold, or at least 100-fold. In another case, the human anti-CCR2 antibodies bind to a polypeptide comprising an amino acid sequence 80, 85, 90, 95, 96, 97, 98, or 99% identical to SEQ ID NO:125.

10 **[00108]** One can determine the selectivity of the anti-CCR2 antibody for CCR2 using methods well known in the art following the teachings of the specification. For instance one can determine the selectivity using Western blot, flow cytometry, ELISA, immunoprecipitation or RIA, and/or functional assays such as chemotaxis, calcium mobilization, or actin polymerization.

15 Methods of Producing Antibodies and Antibody Producing Cell Lines

Immunization

[00109] In some cases, human antibodies are produced by immunizing a non-human, transgenic animal comprising within its genome some or all of human immunoglobulin heavy chain and light chain loci with a CCR2 antigen. In one
20 case, the non-human animal is a XENOMOUSE™ animal. (Amgen Fremont, Inc. (formerly Abgenix, Inc.), Fremont, CA).

[00110] XENOMOUSE™ mice are engineered mouse strains that comprise large fragments of human immunoglobulin heavy chain and light chain loci and are deficient in mouse antibody production. See, e.g., Green *et al.*, *Nature Genetics*
25 7:13-21 (1994) and U.S. Patents 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598, 6,130,364, 6,162,963 and 6,150,584. See also WO 91/10741, WO 94/02602, WO 96/34096, WO 96/33735, WO 98/16654, WO 98/24893, WO 98/50433, WO 99/45031, WO 99/53049, WO 00/09560, and WO 00/037504.

30 **[00111]** In another aspect, provided is a method for making anti-CCR2 antibodies from non-human, non-mouse animals by immunizing non-human transgenic

animals that comprise human immunoglobulin loci with a CCR2 antigen. One can produce such animals using the methods described in the above-cited documents. The methods disclosed in these documents can be modified as described in U.S. Patent 5,994,619, which is hereby incorporated by reference.

5 U.S. Patent 5,994,619 describes methods for producing novel cultured inner cell mass (CICM) cells and cell lines, derived from pigs and cows, and transgenic CICM cells into which heterologous DNA has been inserted. CICM transgenic cells can be used to produce cloned transgenic embryos, fetuses, and offspring. The '619 patent also describes methods of producing transgenic animals that are
10 capable of transmitting the heterologous DNA to their progeny. In preferred cases the non-human animals are mammals, particularly rats, sheep, pigs, goats, cattle or horses.

[00112] XENOMOUSE™ mice produce an adult-like human repertoire of fully human antibodies and generate antigen-specific human antibodies. In some
15 cases, the XENOMOUSE™ mice contain approximately 80% of the human antibody V gene repertoire through introduction of germline configuration fragments of the human heavy chain loci and kappa light chain loci. In other cases, XENOMOUSE™ mice further contain approximately all of the human lambda light chain locus. See Mendez *et al.*, *Nature Genetics* 15:146-156 (1997),
20 Green and Jakobovits, *J. Exp. Med.* 188:483-495 (1998), and WO 98/24893, the disclosures of which are hereby incorporated by reference.

[00113] In another case, the antibodies are generated using VELOCIMOUSE™ technology (Regeneron Pharmaceuticals, Tarrytown, NY.) for immediate generation of genetically altered mice directly from modified embryonic stem (ES)
25 cells (Poueymirou W.T., et al., *Nature Biotechnology* 25:91-99 (2007)).

[00114] In some cases, the non-human animal comprising human immunoglobulin genes are animals that have a human immunoglobulin "minilocus." In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of individual genes from the Ig locus. Thus, one or more V_H
30 genes, one or more D_H genes, one or more J_H genes, a mu constant domain, and a second constant domain (preferably a gamma constant domain) are formed into a construct for insertion into an animal. This approach is described, *inter alia*, in U.S. Patent Nos. 5,545,807, 5,545,806, 5,569,825, 5,625,126, 5,633,425,

5,661,016, 5,770,429, 5,789,650, 5,814,318, 5,591,669, 5,612,205, 5,721,367, 5,789,215, and 5,643,763, hereby incorporated by reference.

[00115] In another aspect, a method for making humanized anti-CCR2 antibodies is provided. In some cases, non-human animals are immunized with a CCR2 antigen as described herein under conditions that permit antibody production. Antibody-producing cells are isolated from the animals, and nucleic acids encoding the heavy and light chains of an anti-CCR2 antibody of interest are isolated. These nucleic acids are subsequently engineered using techniques known to those of skill in the art and as described further below to reduce the amount of non-human sequence, *i.e.*, to humanize the antibody to reduce the immune response in humans

[00116] In some cases, the CCR2 antigen is isolated and/or purified CCR2. In one case, the CCR2 antigen is human CCR2. In some cases, the CCR2 antigen is a fragment of CCR2. In some cases, the CCR2 fragment is an extracellular loop, the N-terminal domain or the C-terminal end of CCR2. In certain cases, the CCR2 fragment comprises the first or second extracellular loop of CCR2. In other cases, the CCR2 fragment comprises the amino acid sequence set forth in SEQ ID NO:128 or SEQ ID NO:129.

[00117] In other cases, the CCR2 fragment does not comprise the third extracellular loop or the N-terminal domain of CCR2.

[00118] In other cases, the CCR2 fragment does not comprise the amino acid sequence set forth in SEQ ID NO:127 or SEQ ID NO:130. In some cases, the CCR2 fragment comprises at least one epitope of CCR2. In other cases, the CCR2 antigen is a cell that expresses or over expresses CCR2 or an immunogenic fragment thereof on its surface. In some cases, the CCR2 antigen is a CCR2 fusion protein. In some cases, the CCR2 is a synthetic peptide immunogen.

[00119] Immunization of animals can be by any method known in the art. See, *e.g.*, Harlow and Lane, Antibodies: A Laboratory Manual, New York: Cold Spring Harbor Press, 1990. Methods for immunizing non-human animals such as mice, rats, sheep, goats, pigs, cattle and horses are well known in the art. See, *e.g.*, Harlow and Lane, *supra*, and U.S. Patent 5,994,619. In one case, the CCR2 antigen is administered with an adjuvant to stimulate the immune response.

Exemplary adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. If a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks. Example 1 exemplifies a method for producing anti-CCR2 monoclonal antibodies in XENOMOUSE™ mice.

10 *Production of Antibodies and Antibody-Producing Cell Lines*

[00120] After immunization of an animal with a CCR2 antigen, antibodies and/or antibody-producing cells can be obtained from the animal. In some cases, anti-CCR2 antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the anti-CCR2 antibodies may be purified from the serum.

[00121] In some cases, antibody-producing immortalized cell lines are prepared from cells isolated from the immunized animal. After immunization, the animal is sacrificed and peripheral blood, lymph node and/or splenic B cells are immortalized by any means known in the art. Methods of immortalizing cells include, but are not limited to, transfecting them with oncogenes, infecting them with an oncogenic virus and cultivating them under conditions that select for immortalized cells, subjecting them to carcinogenic or mutating compounds, fusing them with an immortalized cell, e.g., a myeloma cell, and inactivating a tumor suppressor gene. See, e.g., Harlow and Lane, supra. If fusion with myeloma cells is used, the myeloma cells preferably do not secrete immunoglobulin polypeptides (a non-secretory cell line). Immortalized cells are screened using CCR2, a portion thereof, or a cell expressing CCR2. In one case, the CCR2 portion (i) comprises the first and/or second extracellular loops of CCR2; (ii) comprises the amino acid sequence set forth in SEQ ID NO:128 and/or SEQ ID NO:129; (iii) does not comprise the third extracellular loop and/or the N-terminal domain of CCR2; (iv) does not comprise the amino acid sequence set

forth in SEQ ID NO:127 and/or SEQ ID NO:130; or (v) combinations thereof. In one case, the initial screening is performed using an enzyme-linked immunoassay (ELISA) or a radioimmunoassay. An example of ELISA screening is provided in WO 00/37504, incorporated herein by reference.

- 5 [00122] Anti-CCR2 antibody-producing cells, e.g., hybridomas, are selected, cloned and further screened for desirable characteristics, including robust growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas can be expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*.
- 10 Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

- [00123] In one aspect, the immunized animal is a non-human animal that expresses human immunoglobulin genes and the splenic B cells are fused to a myeloma cell line from the same species as the non-human animal. In an exemplary case, the immunized animal is a XENOMOUSE™ mouse and the myeloma cell line is a non-secretory mouse myeloma. In one case, the myeloma cell line is P3-X63-Ag8.653 (American Type Culture Collection). See, e.g., Example 1.
- 15

- [00124] Screening the immortalized antibody-producing cells to identify an antibody directed to the first and/or second extracellular loop of CCR2 may be achieved by testing if the antibodies produced by the cell bind to a peptide comprising the amino acid sequence of the first or second extracellular loop of CCR2. Alternatively or in combination, antibodies produced by the cell may be tested for binding to chimeric chemokine receptors which have primarily the sequence of another chemokine receptor but sequences of the first and/or second extracellular loops of CCR2. See Example 8, which exemplifies the use of chimeras to map the epitope of anti-CCR2 antibodies. In a complementary case, antibodies produced by the cell which are known to bind to CCR2 may be tested for binding to chimeric chemokine receptors which have primarily the sequence of CCR2 but which lack the wild type first and/or second extracellular loops of CCR2, e.g., have the first and/or second extracellular loops from another cytokine receptor or contain mutations in one or both of these extracellular loops.
- 20
- 25
- 30

[00125] In another aspect, provided are cells and cell lines (including hybridomas) that produce a human anti-CCR2 antibody. In one case, the human anti-CCR2 antibody produced by the cell, cell line or hybridoma is an antagonist of CCR2. In still another case, the human anti-CCR2 antibody (i) binds to the first and/or
5 second extracellular loops of CCR2; (ii) binds to the amino acid sequence set forth in SEQ ID NO:128 and/or SEQ ID NO:129; (iii) does not bind to the third extracellular loop and/or the N-terminal domain of CCR2; (iv) does not bind to the amino acid sequence set forth in SEQ ID NO:127 and/or SEQ ID NO:130; or (v) combinations thereof. In another case, the human anti-CCR2 antibody produced
10 by the cell, cell line or hybridoma does not bind to the third extracellular loop with high affinity, does not bind to the N-terminal domain of CCR2 with high affinity, or does not bind to either with high affinity.

[00126] In still another aspect, a transgenic animal is immunized with CCR2, primary cells, e.g., spleen or peripheral blood cells, are isolated from an
15 immunized transgenic animal and individual cells producing antibodies specific for the desired antigen are identified. For example, polyadenylated mRNA from each individual cell is isolated and reverse transcription polymerase chain reaction (RT-PCR) is performed using sense primers that anneal to variable region sequences, e.g., degenerate primers that recognize most or all of the FR1 regions of human
20 heavy and light chain variable region genes and anti-sense primers that anneal to constant or joining (J) region sequences. cDNAs of the heavy and light chain variable domains are then cloned and expressed in any suitable host cell, e.g., a myeloma cell, as chimeric antibodies with respective immunoglobulin constant regions, such as the heavy chain and κ or λ constant domains. See Babcook,
25 J.S. *et al.*, *Proc. Natl. Acad. Sci. USA* 93: 7843-48 (1996), incorporated herein by reference. Anti-CCR2 antibodies may then be identified and isolated as described herein.

[00127] In another aspect, phage display techniques can be used to provide libraries containing a repertoire of antibodies with varying affinities for CCR2.
30 Primary B cells can be used directly as a source of DNA. The mixture of cDNAs obtained from B cell, e.g., derived from spleens, is used to prepare an expression library, for example, a phage display library transfected into *E.coli*. The resulting cells are tested for immunoreactivity to CCR2. Techniques for the identification of

high affinity human antibodies from such libraries are described by Griffiths *et al.*, *EMBO J.* 13:3245-3260 (1994); Nissim *et al.*, *ibid*, pp. 692-698 and by Griffiths *et al.*, *ibid*, 12:725-734, which are incorporated by reference. Ultimately, clones from the library are identified that produce binding affinities of a desired magnitude for the antigen and the DNA encoding the product responsible for such binding is recovered and manipulated for standard recombinant expression. Phage display libraries may also be constructed using previously manipulated nucleotide sequences and screened in a similar fashion. In general, the cDNAs encoding heavy and light chains are independently supplied or linked to form Fv analogs for production in the phage library.

[00128] The phage library is then screened for the antibodies with the highest affinities for CCR2 and the genetic material recovered from the appropriate clone. Further rounds of screening can increase affinity of the original antibody isolated.

Nucleic Acids, Vectors, Host Cells, and Recombinant Methods of Making Antibodies

Nucleic Acids

[00129] Also encompassed are nucleic acid molecules encoding anti-CCR2 antibodies or antigen-binding portions thereof. In some cases, different nucleic acid molecules encode the heavy chain and the light chain of an anti-CCR2 immunoglobulin. In other cases, the same nucleic acid molecule encodes a heavy chain and a light chain of an anti-CCR2 immunoglobulin. In one case, the nucleic acid encodes a CCR2 antibody, or antigen-binding portion thereof.

[00130] In some cases, the nucleic acid molecule encoding the variable domain of the light chain (V_L) comprises a human V_k1 , V_k2 , V_k3 , V_k4 , V_k5 or V_k6 gene family segment, and a J_k1 , J_k2 , J_k4 , or J_k5 gene segment with or without mutations from the germline.

[00131] In some cases, the nucleic acid molecule encoding the light chain, encodes an amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 substitutions from the germline amino acid sequence(s). In some cases, the nucleic acid molecule comprises a nucleotide sequence that encodes a V_L amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 conservative amino acid substitutions and/or 1, 2, or 3 non-conservative substitutions compared to

germline V_K , and J_K sequences. Substitutions may be in the CDRs, the framework regions, or in the constant domain.

5 [00132] In some cases, the nucleic acid molecule encodes a V_L amino acid sequence comprising one or more variants compared to germline sequence that are identical to the variations found in the V_L of one of the antibodies 4.40, 4.9, 4.22, 4.39 or 4.40 A68G S230P. In some cases, the nucleic acid encodes a V_L amino acid sequence comprising a mutation in one or more of the same positions as mutations from germline in a CCR2 antibody provided herein but that comprises a different substitution, in some cases a conservative substitution,
10 compared to the substitution in the provided antibody.

[00133] In some cases, the nucleic acid molecule encodes at least three amino acid substitutions compared to the germline sequence found in the V_L of one of the antibodies 4.40, 4.9, 4.22, 4.39 or 4.40 A68G S230P.

15 [00134] In some cases, the nucleic acid molecule comprises a nucleotide sequence that encodes the V_L amino acid sequence of monoclonal antibody 4.40 (SEQ ID NO:101), 4.9 (SEQ ID NO:29), 4.22 (SEQ ID NO:65), 4.39 (SEQ ID NO:194) or 4.40 A68G S230P (SEQ ID NO:113), or a variant or portion thereof. In some cases, the nucleic acid encodes an amino acid sequence comprising the light chain CDRs of one of said above-listed antibodies. In some cases, said
20 portion is a contiguous portion comprising CDR1-CDR3.

[00135] In some cases, the nucleic acid molecule encodes a V_L amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to a V_L amino acid sequence of any one of a V_L region of antibodies 4.40, 4.9, 4.22, 4.39 or 4.40 A68G S230P, or an amino acid sequence of a V_L region of
25 any one of SEQ ID NO:101, SEQ ID NO:113, SEQ ID NO:29, SEQ ID NO:194 or SEQ ID NO:65. Nucleic acid molecules include nucleic acids encoding the V_L region found in SEQ ID NO:101, SEQ ID NO:113, SEQ ID NO:29 or SEQ ID NO:65.

30 [00136] In another case, the nucleic acid encodes a full-length light chain of an antibody selected from 4.40, 4.9, 4.22, 4.39 or 4.40 A68G S230P, or a light chain comprising the amino acid sequence of SEQ ID NO:100, SEQ ID NO:112, SEQ ID NO:64, SEQ ID NO:193 or SEQ ID NO:28, or said light chain sequence comprising a mutation, such as one disclosed herein.

[00137] In still another case, the nucleic acid molecule encodes the variable domain of the heavy chain (V_H) that comprises a human 3-30 or 1-46 V_H gene sequence or a sequence derived there from. In various cases, the nucleic acid molecule utilizes a human 3-30 V_H gene sequence, a human D1-7 gene sequence and a human J_H3B gene sequence; a human 1-46 V_H gene sequence, a human D1-7 gene sequence and a human J_H3B gene sequence; or sequence derived from the human genes.

[00138] In some cases, the nucleic acid molecule encodes an amino acid sequence comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 or 18 mutations compared to the germline amino acid sequence of the human V, D or J genes. In some cases, said mutations are in the V_H region. In some cases, said mutations are in the CDRs.

[00139] In some cases, the nucleic acid molecule encodes an amino acid sequence comprising one or more amino acid mutations compared to the germline sequence that are identical to amino acid mutations found in the V_H of monoclonal antibody 4.40, 4.22, 4.39 or 4.9. In some cases, the nucleic acid encodes at least three amino acid mutations compared to the germline sequences that are identical to at least three amino acid mutations found in one of the above-listed monoclonal antibodies.

[00140] In some cases, the nucleic acid molecule comprises a nucleotide sequence that encodes at least a portion of the V_H amino acid sequence of a monoclonal antibody selected from 4.40 (SEQ ID NO:83), 4.22 (SEQ ID NO:47), 4.39 (SEQ ID NO:176) or 4.9 (SEQ ID NO:11), a variant thereof, or said sequence having conservative amino acid mutations and/or a total of three or fewer non-conservative amino acid substitutions. In various cases the sequence encodes one or more CDRs, a CDR3 region, all three CDRs, a contiguous portion including CDR1-CDR3, or the entire V_H region, with or without a signal sequence.

[00141] In some cases, the nucleic acid molecule comprises a nucleotide sequence that encodes the amino acid sequence of one of SEQ ID NO:82, SEQ ID NO:46, SEQ ID NO:10, SEQ ID NO:175 or SEQ ID NO:116, or said sequence having a signal sequence. In some preferred cases, the nucleic acid molecule comprises at least a portion of the nucleotide sequence of SEQ ID NO:73 or SEQ ID NO:115, or said sequence having a signal sequence. In some cases, said

portion encodes the V_H region (with or without a signal sequence), a CDR3 region, all three CDRs, or a contiguous region including CDR1-CDR3.

[00142] In some cases, the nucleic acid molecule encodes a V_H amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical to the V_H amino acid sequences of any one of V_H region of antibodies 4.40, 4.9, 4.22, 4.39 or 4.40 A68G S230P, or an amino acid sequence of a V_H region of any one of SEQ ID NO:83, SEQ ID NO:47, SEQ ID NO:176 or SEQ ID NO:11. Also included are nucleic acids encoding the amino acid sequence of 4.40 (SEQ ID NO:83), 4.22 (SEQ ID NO:47), 4.39 (SEQ ID NO:176) or 4.9 (SEQ ID NO:11), or to a V_H region thereof, or that has the nucleotide sequence of SEQ ID NO:74, SEQ ID NO:38, SEQ ID NO:167, SEQ ID NO:2 or that encodes a V_H region thereof.

[00143] In another case, the nucleic acid encodes a full-length heavy chain of an antibody selected from 4.40, 4.22, 4.9, 4.39, or 4.40 A68G S230P, or a heavy chain having the amino acid sequence of SEQ ID NO:10, SEQ ID NO:46, SEQ ID NO:82, SEQ ID NO:175 or SEQ ID NO:116, with or without a signal sequence, or a heavy chain comprising a mutation, such as one of the variants discussed herein. Further, the nucleic acid may comprise the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:37, SEQ ID NO:73, SEQ ID NO:166 or SEQ ID NO:115, or a nucleic acid molecule encoding a heavy chain comprising a mutation, such as one of the variants discussed herein.

[00144] A nucleic acid molecule encoding the heavy or light chain of an anti-CCR2 antibody or portions thereof can be isolated from any source that produces such antibody. In various cases, the nucleic acid molecules are isolated from a B cell isolated from an animal immunized with CCR2 or from an immortalized cell derived from such a B cell that expresses an anti-CCR2 antibody. Methods of isolating mRNA encoding an antibody are well-known in the art. See, e.g., Sambrook *et al.* The mRNA may be used to produce cDNA for use in the polymerase chain reaction (PCR) or cDNA cloning of antibody genes. In one case, the nucleic acid molecule is isolated from a hybridoma that has as one of its fusion partners a human immunoglobulin-producing cell from a non-human transgenic animal. In other cases, the human immunoglobulin producing cell is isolated from a XENOMOUSE™ animal. In another case, the human

immunoglobulin-producing cell is from a non-human, non-mouse transgenic animal, as described herein. In another case, the nucleic acid is isolated from a non-human, non-transgenic animal. The nucleic acid molecules isolated from a non-human, non-transgenic animal may be used, e.g., for humanized antibodies.

5 **[00145]** In some cases, a nucleic acid encoding a heavy chain of an anti-CCR2 antibody comprises a nucleotide sequence encoding a V_H domain joined in-frame to a nucleotide sequence encoding a heavy chain constant domain from any source. Similarly, a nucleic acid molecule encoding a light chain of an anti-CCR2 antibody can comprise a nucleotide sequence encoding a V_L domain joined in-
10 frame to a nucleotide sequence encoding a light chain constant domain from any source.

[00146] In a further aspect, nucleic acid molecules encoding the variable domain of the heavy (V_H) and/or light (V_L) chains are "converted" to full-length antibody genes. In one case, nucleic acid molecules encoding the V_H or V_L domains are
15 converted to full-length antibody genes by insertion into an expression vector already encoding heavy chain constant (C_H) or light chain constant (C_L) domains, respectively, such that the V_H segment is operatively linked to the C_H segment(s) within the vector, and/or the V_L segment is operatively linked to the C_L segment within the vector. In another case, nucleic acid molecules encoding the V_H and/or
20 V_L domains are converted into full-length antibody genes by linking, e.g., ligating, a nucleic acid molecule encoding a V_H and/or V_L domains to a nucleic acid molecule encoding a C_H and/or C_L domain using standard molecular biological techniques. Nucleotide sequences of human heavy and light chain immunoglobulin constant domain genes are known in the art. See, e.g., Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed., NIH Publ. No. 91-
25 3242, 1991. Nucleic acid molecules encoding the full-length heavy and/or light chains may then be expressed from a cell into which they have been introduced and the anti-CCR2 antibody isolated.

[00147] The nucleic acid molecules may be used to recombinantly express anti-
30 CCR2 antibodies. The nucleic acid molecules also may be used to produce chimeric antibodies, bispecific antibodies, single chain antibodies, immunoadhesins, diabodies, mutated antibodies and antibody derivatives, as described further below. If the nucleic acid molecules are derived from a non-

human, non-transgenic animal, the nucleic acid molecules may be used for antibody humanization, also as described herein.

[00148] In some cases, nucleic acids encoding a full-length heavy chain or a full-length light chain wherein the nucleotide sequence encoding the constant region amino acid sequence contain one or more mutations compared to a germline human constant region sequence are provided. For example, the nucleic acid may encode an amino acid substitution that improves a property of the antibody, by adding or removing a glycosylation site or encoding a substitutions that improves the stability or half-life of the antibody. The nucleic acid also may contain "silent" mutations to add or remove a restriction enzyme site, for example to facilitate cloning of the nucleic acid into a particular expression vector.

Vectors

[00149] Vectors are provided comprising nucleic acid molecules that encode the heavy chain and/or light chain of an anti-CCR2 antibody or an antigen-binding portions thereof. Also provided are vectors comprising nucleic acid molecules encoding fusion proteins, modified antibodies, antibody fragments, and probes thereof.

[00150] In some cases, the anti-CCR2 antibodies or antigen-binding portions are expressed by inserting DNAs encoding partial or full-length light and/or heavy chains, obtained as described herein, into expression vectors such that the genes are operatively linked to necessary expression control sequences such as transcriptional and translational control sequences. Expression vectors include plasmids, retroviruses, adenoviruses, adeno-associated viruses (AAV), plant viruses such as cauliflower mosaic virus, tobacco mosaic virus, cosmids, YACs, EBV derived episomes, and the like. In some instances the antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be inserted into separate vectors or into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (e.g., ligation of

complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present).

5 [00151] A convenient vector is one that encodes a functionally complete human C_H or C_L immunoglobulin sequence, with appropriate restriction sites engineered so that any V_H or V_L sequence can be inserted and expressed, as described herein. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C domain, and also at the splice regions that occur within the human C_H exons. Polyadenylation and transcription termination occur at native chromosomal sites
10 downstream of the coding regions. The recombinant expression vector also can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene may be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the immunoglobulin chain. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal
15 peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

[00152] In addition to the antibody chain genes, the recombinant expression vectors carry regulatory sequences that control the expression of the antibody chain genes in a host cell. It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences
20 may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from retroviral LTRs, cytomegalovirus (CMV) (such as the CMV
25 promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)), polyoma and strong mammalian promoters such as native immunoglobulin and actin promoters. For further description of viral regulatory elements, and sequences thereof, see *e.g.*, U.S. Patent No. 5,168,062, U.S.
30 Patent No. 4,510,245 and U.S. Patent No. 4,968,615. Methods for expressing antibodies in plants, including a description of promoters and vectors, as well as transformation of plants is known in the art. See, *e.g.*, United States Patent

6,517,529, incorporated herein by reference. Methods of expressing polypeptides in bacterial cells or fungal cells, e.g., yeast cells, are also well known in the art.

[00153] In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors may carry additional sequences, such as

- 5 sequences that regulate replication of the vector in host cells (e.g., origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see e.g., U.S. Patent Nos. 4,399,216, 4,634,665 and 5,179,017, incorporated herein by reference). For example, typically the selectable marker gene confers resistance
- 10 to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr-host cells with methotrexate selection/amplification), the neo gene (for G418 selection), and the glutamate synthetase (GS) gene.

15 *Non-Hybridoma Host Cells and Methods of Recombinantly Producing Protein*

[00154] Nucleic acid molecules encoding anti-CCR2 antibodies and vectors comprising these nucleic acid molecules can be used for transfection of a suitable mammalian, plant, bacterial, insect or yeast host cell. Transformation can be by any known method for introducing polynucleotides into a host cell. Methods for

- 20 introduction of heterologous polynucleotides into mammalian cells are well known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene-mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei. In addition, nucleic acid molecules may be introduced into
- 25 mammalian cells by viral vectors. Methods of transforming cells are well known in the art. See, e.g., U.S. Patent Nos. 4,399,216, 4,912,040, 4,740,461, and 4,959,455, incorporated herein by reference). Methods of transforming plant cells are well known in the art, including, e.g., Agrobacterium-mediated transformation, biolistic transformation, direct injection, electroporation and viral transformation.
- 30 Methods of transforming bacterial, insect cells, and yeast cells are also well known in the art.

[00155] Mammalian cell lines available as hosts for expression are well known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC). These include, *inter alia*, Chinese hamster ovary (CHO) cells, NS0 cells, NSO cells, SP2 cells, HEK-293T cells, NIH-3T3 cells, 5 HeLa cells, baby hamster kidney (BHK) cells, African green monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), A549 cells, and a number of other cell lines. Cell lines of particular preference are selected through determining which cell lines have high expression levels. Other cell lines that may be used are insect cell lines, such as Sf9 or Sf21 cells. When recombinant 10 expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification 15 methods. Plant host cells include, e.g., *Nicotiana*, *Arabidopsis*, duckweed, corn, wheat, potato, etc. Bacterial host cells include *E. coli* and *Streptomyces* species. Yeast host cells include *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae* and *Pichia pastoris*.

[00156] Further, expression of antibodies from production cell lines can be 20 enhanced using a number of known techniques. For example, the glutamine synthetase gene expression system (the GS system) is a common approach for enhancing expression under certain conditions. The GS system is discussed in whole or part in connection with European Patent Nos. 216 846, 256 055, 323 997 and 338 841.

25 [00157] It is likely that antibodies expressed by different cell lines or in transgenic animals will have different glycosylation from each other. However, all antibodies encoded by the nucleic acid molecules provided herein, or comprising the amino acid sequences provided herein are part of the instant disclosure, regardless of the glycosylation of the antibodies.

30 *Transgenic Animals and Plants*

[00158] Anti-CCR2 antibodies also can be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy

and light chain sequences of interest and production of the antibody in a recoverable form there from. In connection with the transgenic production in mammals, anti-CCR2 antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. See, e.g., U.S. Patent Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957, incorporated herein by reference. In some cases, non-human transgenic animals that comprise human immunoglobulin loci are immunized with CCR2 or an immunogenic portion thereof, as described herein. Methods for making antibodies in plants are described, e.g., in U.S. patents 6,046,037 and 5,959,177, incorporated herein by reference.

10 **[00159]** In some cases, non-human transgenic animals or plants are produced by introducing one or more nucleic acid molecules encoding an anti-CCR2 antibody into the animal or plant by standard transgenic techniques. See Hogan and United States Patent 6,417,429, *supra*. The transgenic cells used for making the transgenic animal can be embryonic stem cells or somatic cells or a fertilized egg.

15 The transgenic non-human organisms can be chimeric, nonchimeric heterozygotes, and nonchimeric homozygotes. See, e.g., Hogan *et al.*, Manipulating the Mouse Embryo: A Laboratory Manual second ed., Cold Spring Harbor Press (1999); Jackson *et al.*, Mouse Genetics and Transgenics: A Practical Approach, Oxford University Press (2000); and Pinkert, Transgenic

20 Animal Technology: A Laboratory Handbook, Academic Press (1999), all incorporated herein by reference. In some cases, the transgenic non-human animals have a targeted disruption and replacement by a targeting construct that encodes a heavy chain and/or a light chain of interest. In one case, the transgenic animals comprise and express nucleic acid molecules encoding heavy

25 and light chains that specifically bind to CCR2, and preferably (i) bind to the first and/or second extracellular loops of CCR2; (ii) which do not bind to the N-terminal end or the third extracellular loop of CCR2 or both; or (iii) both. In one case, the transgenic animals comprise and express nucleic acid molecules encoding heavy and light chains that specifically bind to human CCR2. In some cases, the

30 transgenic animals comprise nucleic acid molecules encoding a modified antibody such as a single-chain antibody, a chimeric antibody or a humanized antibody. The anti-CCR2 antibodies may be made in any transgenic animal. In one case, the non-human animals are mice, rats, sheep, pigs, goats, cattle or horses. The

non-human transgenic animal expresses said encoded polypeptides in blood, milk, urine, saliva, tears, mucus and other bodily fluids.

Phage Display Libraries

[00160] A method is provided for producing an anti-CCR2 antibody or antigen-binding portion thereof comprising the steps of synthesizing a library of human antibodies on phage, screening the library with CCR2 or a portion thereof, isolating phage that bind CCR2, and obtaining the antibody from the phage. By way of example, one method for preparing the library of antibodies for use in phage display techniques comprises the steps of immunizing a non-human animal comprising human immunoglobulin loci with CCR2 or an antigenic portion thereof to create an immune response, extracting antibody-producing cells from the immunized animal; isolating RNA encoding heavy and light chains of antibodies from the extracted cells, reverse transcribing the RNA to produce cDNA, amplifying the cDNA using primers, and inserting the cDNA into a phage display vector such that antibodies are expressed on the phage. Recombinant anti-CCR2 antibodies may be obtained in this way.

[00161] Recombinant anti-CCR2 human antibodies can be isolated by screening a recombinant combinatorial antibody library. The library may be a scFv phage display library, generated using human V_L and V_H cDNAs prepared from mRNA isolated from B cells. Methods for preparing and screening such libraries are known in the art. Kits for generating phage display libraries are commercially available (e.g., the Pharmacia Recombinant Phage Antibody System, catalog no. 27-9400-01; and the Stratagene SurfZAP™ phage display kit, catalog no. 240612). There also are other methods and reagents that can be used in generating and screening antibody display libraries (see, e.g., U.S. Patent No. 5,223,409; PCT Publication Nos. WO 92/18619, WO 91/17271, WO 92/20791, WO 92/15679, WO 93/01288, WO 92/01047, WO 92/09690; Fuchs *et al.*, *Bio/Technology* 9:1370-1372 (1991); Hay *et al.*, *Hum. Antibod. Hybridomas* 3:81-85 (1992); Huse *et al.*, *Science* 246:1275-1281 (1989); McCafferty *et al.*, *Nature* 348:552-554 (1990); Griffiths *et al.*, *EMBO J.* 12:725-734 (1993); Hawkins *et al.*, *J. Mol. Biol.* 226:889-896 (1992); Clackson *et al.*, *Nature* 352:624-628 (1991); Gram *et al.*, *Proc. Natl. Acad. Sci. USA* 89:3576-3580 (1992); Garrad *et al.*, *Bio/Technology*

9:1373-1377 (1991); Hoogenboom *et al.*, *Nuc. Acid Res.* 19:4133-4137 (1991); and Barbas *et al.*, *Proc. Natl. Acad. Sci. USA* 88:7978-7982 (1991), all incorporated herein by reference.

[00162] In one case, to isolate and produce human anti-CCR2 antibodies with the
5 desired characteristics, a human anti-CCR2 antibody as described herein is first
used to select human heavy and light chain sequences having similar binding
activity toward CCR2, using the epitope imprinting methods described in PCT
Publication No. WO 93/06213, incorporated herein by reference. The antibody
libraries used in this method may be scFv libraries prepared and screened as
10 described in PCT Publication No. WO 92/01047, McCafferty *et al.*, *Nature*
348:552-554 (1990); and Griffiths *et al.*, *EMBO J.* 12:725-734 (1993), all
incorporated herein by reference. The scFv antibody libraries can be screened
using human CCR2 as the antigen.

[00163] Once initial human V_L and V_H domains are selected, "mix and match"
15 experiments are performed, in which different pairs of the initially selected V_L and
V_H segments are screened for CCR2 binding to select preferred V_L/V_H pair
combinations. Additionally, to further improve the quality of the antibody, the V_L
and V_H segments of the preferred V_L/V_H pair(s) can be randomly mutated,
preferably within the CDR3 region of V_H and/or V_L, in a process analogous to the
20 in vivo somatic mutation process responsible for affinity maturation of antibodies
during a natural immune response. This in vitro affinity maturation can be
accomplished by amplifying V_H and V_L domains using PCR primers
complimentary to the V_H CDR3 or V_L CDR3, respectively, which primers have
been "spiked" with a random mixture of the four nucleotide bases at certain
25 positions such that the resultant PCR products encode V_H and V_L segments into
which random mutations have been introduced into the V_H and/or V_L CDR3
regions. These randomly mutated V_H and V_L segments can be re-screened for
binding to CCR2.

[00164] Following screening and isolation of an anti-CCR2 antibody from a
30 recombinant immunoglobulin display library, nucleic acids encoding the selected
antibody can be recovered from the display package (e.g., from the phage
genome) and subcloned into other expression vectors by standard recombinant
DNA techniques. If desired, the nucleic acid can further be manipulated to create

other antibody forms, as described herein. To express a recombinant human antibody isolated by screening of a combinatorial library, the DNA encoding the antibody is cloned into a recombinant expression vector and introduced into a mammalian host cells, as described herein.

5 *Class switching*

[00165] Another aspect provides a method for converting the class or subclass of an anti-CCR2 antibody to another class or subclass. In some cases, a nucleic acid molecule encoding a V_L or V_H that does not include sequences encoding C_L or C_H is isolated using methods well-known in the art. The nucleic acid molecule
10 then is operatively linked to a nucleotide sequence encoding a C_L or C_H from a desired immunoglobulin class or subclass. This can be achieved using a vector or nucleic acid molecule that comprises a C_L or C_H chain, as described herein. For example, an anti-CCR2 antibody that was originally IgM can be class switched to an IgG. Further, the class switching may be used to convert one IgG
15 subclass to another, e.g., from IgG1 or IgG2 to IgG4. Another method for producing an antibody comprising a desired isotype comprises the steps of isolating a nucleic acid encoding a heavy chain of an anti-CCR2 antibody and a nucleic acid encoding a light chain of an anti-CCR2 antibody, isolating the sequence encoding the V_H region, ligating the V_H sequence to a sequence
20 encoding a heavy chain constant domain of the desired isotype, expressing the light chain gene and the heavy chain construct in a cell, and collecting the anti-CCR2 antibody with the desired isotype.

Deimmunized Antibodies

[00166] In another aspect, the antibody may be deimmunized to reduce its
25 immunogenicity using the techniques described in, e.g., PCT Publication Nos. WO98/52976 and WO00/34317 (incorporated herein by reference).

Mutated Antibodies

[00167] In another aspect, the nucleic acid molecules, vectors and host cells may be used to make mutated anti-CCR2 antibodies. The antibodies may be mutated
30 in the variable domains of the heavy and/or light chains, e.g., to alter a binding property of the antibody. For example, a mutation may be made in one or more of

the CDRs to increase or decrease the K_D of the antibody for CCR2, to increase or decrease k_{off} , or to alter the binding specificity of the antibody. Techniques in site-directed mutagenesis are well-known in the art. See, e.g., Sambrook *et al.* and Ausubel *et al.*, supra. In another case, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in monoclonal antibody 4.40, 4.9, 4.22, 4.39 or 4.40 A68G S230P. The mutations may be made in a CDR or framework region of a variable domain, or in a constant domain. In one case, the mutations are made in a variable domain. In some cases, one or more mutations are made at an amino acid residue that is known to be changed compared to the germline in a CDR or framework region of a variable domain of an amino acid sequence selected from SEQ ID NO:11, SEQ ID NO:47, SEQ ID NO:83, SEQ ID NO:176, SEQ ID NO:29, SEQ ID NO:65, SEQ ID NO:101, SEQ ID NO:194 or SEQ ID NO:113.

[00168] In another aspect, the framework region is mutated so that the resulting framework region(s) have the amino acid sequence of the corresponding germline gene. A mutation may be made in a framework region or constant domain to increase the half-life of the anti-CCR2 antibody. See, e.g., PCT Publication No. WO 00/09560, incorporated herein by reference. A mutation in a framework region or constant domain also can be made to alter or reduce the immunogenicity of the antibody, to provide a site for covalent or non-covalent binding to another molecule, to add or remove one or more glycosylation sites or to alter such properties as complement fixation, FcR binding and antibody-dependent cell-mediated cytotoxicity (ADCC). A single antibody may have mutations in any one or more of the CDRs or framework regions of the variable domain or in the constant domain.

[00169] In some cases, there are from 1 to 8, including any number in between, amino acid mutations in either the V_H or V_L domains of the mutated anti-CCR2 antibody compared to the anti-CCR2 antibody prior to mutation. In any of the above, the mutations may occur in one or more CDRs. Further, any of the mutations can be conservative amino acid substitutions. In some cases, there are no more than 5, 4, 3, 2, or 1 amino acid change in the constant domains.

Modified Antibodies

- [00170] In another aspect, a fusion antibody or immunoadhesin may be made that comprises all or a portion of an anti-CCR2 antibody linked to another polypeptide. In one case, only the variable domains of the anti-CCR2 antibody are linked to the polypeptide. In still another case, the V_H domain of an anti-CCR2 antibody is linked to a first polypeptide, while the V_L domain of an anti-CCR2 antibody is linked to a second polypeptide that associates with the first polypeptide in a manner such that the V_H and V_L domains can interact with one another to form an antigen binding site. In still another case, the V_H domain is separated from the V_L domain by a linker such that the V_H and V_L domains can interact with one another (see below under Single Chain Antibodies). The V_H-linker-V_L antibody is then linked to the polypeptide of interest. The fusion antibody is useful for directing a polypeptide to a CCR2-expressing cell or tissue. The polypeptide may be a therapeutic agent, such as a toxin, chemokine or other regulatory protein, or may be a diagnostic agent, such as an enzyme that may be easily visualized, such as horseradish peroxidase. In addition, fusion antibodies can be created in which two (or more) single-chain antibodies are linked to one another. This is useful if one wants to create a divalent or polyvalent antibody on a single polypeptide chain, or if one wants to create a bispecific antibody.
- [00171] To create a single chain antibody (scFv), the V_H- and V_L-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g., encoding the amino acid sequence (Gly₄-Ser)₃, such that the V_H and V_L sequences can be expressed as a contiguous single-chain protein, with the V_L and V_H domains joined by the flexible linker. See, e.g., Bird *et al.*, *Science* 242:423-426 (1988); Huston *et al.*, *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988); McCafferty *et al.*, *Nature* 348:552-554 (1990). The single chain antibody may be monovalent, if only a single V_H and V_L are used, bivalent, if two V_H and V_L are used, or polyvalent, if more than two V_H and V_L are used. Bispecific or polyvalent antibodies may be generated that bind specifically to CCR2 and to another molecule.
- [00172] In another aspect, other modified antibodies may be prepared using anti-CCR2 antibody encoding nucleic acid molecules. For instance, "Kappa bodies" (Ill *et al.*, *Protein Eng.* 10: 949-57 (1997)), "Minibodies" (Martin *et al.*, *EMBO J.* 13:

5303-9 (1994)), "Diabodies" (Holliger *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993)), or "Janusins" (Traunecker *et al.*, *EMBO J.* 10:3655-3659 (1991) and Traunecker *et al.*, *Int. J. Cancer* (Suppl.) 7:51-52 (1992)) may be prepared using standard molecular biological techniques following the teachings of the

5 specification.

[00173] Bispecific antibodies or antigen-binding fragments can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments.

See, *e.g.*, Songsivilaj & Lachmann, *Clin. Exp. Immunol.* 79: 315-321 (1990),

Kostelny *et al.*, *J. Immunol.* 148:1547-1553 (1992). In addition, bispecific

10 antibodies may be formed as "diabodies" or "Janusins." In some cases, the bispecific antibody binds to two different epitopes of CCR2. In some cases, the bispecific antibody has a first heavy chain and a first light chain from monoclonal antibody 4.40, 4.9 or 4.40 A68G S230P and an additional antibody heavy chain and light chain. In some cases, the additional light chain and heavy chain also
15 are from one of the above-identified monoclonal antibodies, but are different from the first heavy and light chains.

[00174] In some cases, the modified antibodies described herein are prepared using one or more of the variable domains or CDRs from a human anti-CCR2 monoclonal antibody provided herein.

20 *Derivatized and Labeled Antibodies*

[00175] An anti-CCR2 antibody or antigen-binding portion can be derivatized or linked to another molecule (*e.g.*, another peptide or protein). In general, the antibodies or portion thereof are derivatized such that the CCR2 binding is not affected adversely by the derivatization or labeling. Accordingly, the antibodies
25 and antibody portions are intended to include both intact and modified forms of the human anti-CCR2 antibodies described herein. For example, an antibody or antibody portion can be functionally linked (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (*e.g.*, a bispecific antibody or a diabody), a detection
30 agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

[00176] One type of derivatized antibody is produced by crosslinking two or more antibodies (of the same type or of different types, e.g., to create bispecific antibodies). Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, IL.

[00177] Another type of derivatized antibody is a labeled antibody. Useful detection agents with which an antibody or antigen-binding portion may be derivatized include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, phycoerythrin, 5-dimethylamine-1-naphthalenesulfonyl chloride, lanthanide phosphors and the like. An antibody can also be labeled with enzymes that are useful for detection, such as horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like.

When an antibody is labeled with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody can also be labeled with biotin, and detected through indirect measurement of avidin or streptavidin binding. An antibody can also be labeled with a predetermined polypeptide epitope recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some cases, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.

[00178] An anti-CCR2 antibody can also be labeled with a radiolabeled amino acid. The radiolabel can be used for both diagnostic and therapeutic purposes. For instance, the radiolabel can be used to detect CCR2-expressing tumors by X-ray or other diagnostic techniques. Further, the radiolabel can be used therapeutically as a toxin for cancerous cells or tumors. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionuclides -- ^3H , ^{14}C , ^{15}N , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , and ^{131}I .

[00179] An anti-CCR2 antibody can also be derivatized with a chemical group such as polyethylene glycol (PEG), a methyl or ethyl group, or a carbohydrate group. These groups are useful to improve the biological characteristics of the antibody, e.g., to increase serum half-life or to increase tissue binding.

5 [00180] In some cases, the anti-CCR2 antibody can be labeled with a paramagnetic, radioactive or fluorogenic ion or moiety that is detectable upon imaging.

[00181] In some cases, the paramagnetic ion is chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III),
10 ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III) or erbium (III). In other cases, the radioactive ion is iodine123, technetium99, indium111, rhenium188, rhenium186, copper67, iodine131, yttrium90, iodine125, astatine211, and gallium67. In other cases, the anti-CCR2 antibody is labeled with an X-ray imaging agent such as lanthanum (III), gold (III)
15 lead (II) and bismuth (III).

Pharmaceutical Compositions and Kits

[00182] Compositions comprising a human anti-CCR2 antibody with antagonist properties are provided. Such compositions are useful to treat a condition in which CCR2 has a role, including, but not limited to, liver fibrosis, renal fibrosis,
20 pulmonary fibrosis, psoriasis; inflammatory disorders, allergic disorders, autoimmune diseases, graft rejection disorders, atherosclerosis, obesity, HIV infection, neuropathic pain, inflammation associated with ischemia, stenosis and restenosis, cancer, sepsis, scleroderma, and diabetes. In some cases, the treatment is of liver fibrosis mediated by hepatitis C virus (HCV), hepatitis B virus
25 (HBV), non-alcohol steatohepatitis (NASH), and /or alcohol induced steatohepatitis (ASH). In some cases, the subject is in need of a reduction of leukocyte infiltration into tissues, such as tissues that are the sites of inflammatory responses. In some cases, the subject of treatment is a human. In other cases, the subject is a veterinary subject. Examples of tissues in need of reduced
30 inflammation or reduced leukocyte infiltration include but are not limited to, connective tissue, cartilage, liver, lung, kidney, neural tissue including brain, spinal cord, and peripheral neural tissue, heart, blood vessels, esophagus,

stomach, small intestine, large intestine, colon, prostate, pancreas, urinary tract, ovaries, breasts, uterus, testis, penis, bone, muscle, thyroid gland, adrenal gland, pituitary, adipose tissue, bone marrow, blood, thymus, spleen, lymph nodes, skin, eye, ear or nose. In one case, the tissues are tissues having mucosal surfaces.

5 **[00183]** As used herein, "pharmaceutically acceptable carrier" means any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Some examples of pharmaceutically acceptable carriers merely by way of illustration, are water, saline, phosphate buffered saline, dextrose, glycerol,
10 ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Additional examples of pharmaceutically acceptable substances are wetting agents or minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or
15 buffers, which enhance the shelf life or effectiveness of the antibody.

[00184] The compositions may be in a variety of forms, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The form depends on the intended mode of administration and
20 therapeutic application. Typical compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In one case, the antibody is administered by intravenous infusion or injection. In still another case,
25 the antibody is administered by intramuscular or subcutaneous injection.

[00185] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by
30 incorporating the anti-CCR2 antibody in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic

dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional
5 desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that
10 delays absorption, for example, monostearate salts and gelatin.

[00186] The antibodies may be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous, intramuscular, or intravenous infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will
15 vary depending upon the desired results. Other modes of administration include intraperitoneal, intrabronchial, transmucosal, intraspinal, intrasynovial, intraaortic, intranasal, ocular, otic, topical and buccal, and intratumor.

[00187] In certain cases, the active compound of the antibody compositions may be prepared with a carrier that will protect the antibody against rapid release, such
20 as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art.

25 See, e.g., Sustained and Controlled Release Drug Delivery Systems (J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978).

[00188] Additional active compounds also can be incorporated into the compositions. In certain cases, an inhibitory anti-CCR2 antibody is co-formulated with and/or co-administered with one or more additional therapeutic, diagnostic, or
30 prophylactic agents. Therapeutic agents include, without limitation, an anti-CCR2 antibody with a different fine specificity, antibodies that bind other targets, photosensitizers, androgen, estrogen, nonsteroidal anti-inflammatory agents, antihypertensive agents, analgesic agents, antidepressants, antibiotics, anti-

cancer agents, anesthetics, anti-emetics, anti-infectants, contraceptives, anti-diabetic agents, steroids, anti-allergy agents, chemotherapeutic agents, anti-migraine agents, agents for smoking cessation, anti-viral agents, immunosuppressants, thrombolytic agent, cholesterol-lowering agents and anti-obesity agents.

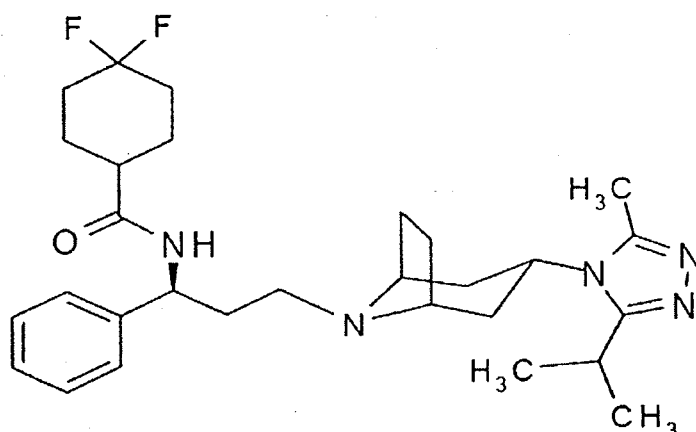
5
[00189] Therapeutic agents also include peptide analogues that inhibit CCR2, antibodies or other molecules that bind to MCP-1, MCP-2, MCP-3 or MCP-4 and prevent their binding to CCR2, and agents that inhibit CCR2 expression. In one case, the additional agents that inhibit CCR2 expression comprise an antisense
10 nucleic acid capable of hybridizing to a CCR2 mRNA, such as a hairpin RNA or siRNA. Sequence-specific nucleic acids capable of inhibiting gene function by RNA interference are well-known in the art. Such combination therapies may require lower dosages of the inhibitory anti-CCR2 antibody as well as the co-administered agents, thus avoiding possible toxicities or complications associated
15 with the various monotherapies.

[00190] In certain specific cases, the therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is an antimicrobial agent. Antimicrobial agents include antibiotics (e.g. antibacterial), antiviral agents, antifungal agents, and anti-protozoan agents. Non-limiting
20 examples of antimicrobial agents are sulfonamides, trimethoprim-sulfamethoxazole, quinolones, penicillins, and cephalosporins.

[00191] In certain specific cases, the therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody and a chemokine antagonist, a CCR2 antagonist, a MCP-1 antagonist or a CCR5
25 antagonist. The CCR2 antagonist or a MCP-1 antagonist include, but are not limited to: antibodies directed to MCP-1 (US Patent 7,202,343, US2005/0025768, US2006/0039913, US2006/0246069, & US2004/004/0047860); tetrahydropyranyl cyclopentyl benzylamide compounds (US2006/0116421); heteroaryl piperidine compounds (US2005/0250781); piperidinyl cyclopentyl aryl benzylamide
30 compounds (US2006/0173013); cyclic amine compounds (US Patent 6,140,349 & US Patent 6,476,054); cyclopentyl compounds (US2002/0049222 & US Patent 6,545,023); tetrahydropyranyl cyclopentyl tetrahydropyridopyridine compounds (US2004/0167156, US Patent 6,812,234 & US Patent 7,230,008);

aminocyclopentyl fused heterotricyclicamide compounds (US2007/0004714);
 piperidinyl- α -aminoamide compounds (US2005/0250814); substituted
 pyrazole compounds (WO06/88813); tetrahydropyranyl cyclopentyl heterocyclic
 amide compounds (US2006/0178363); 7 and 8 membered heterocyclic
 5 cyclopentyl benzamide compounds (US2006/0183731); benzoxazinyl-
 amidocyclopentyl-heterocyclic compounds (US2006/0069088); 3-
 aminocyclopentanecarboxamide compounds (US2007/0149532); nitrogen
 containing heterocyclic compounds (US2007/0155713); triazolyl phenyl
 bensesulonamide compounds (WO08/10934); substituted pyrrolidine derivatives
 10 (US2004/0186140); substituted benzamide and substituted phenylcarbamate
 derivatives (US Patent 7,087,604); substituted cycloalkylamine compounds
 (US2005/0054626); substituted bicycloalkylamine compounds (US2005/0227960);
 pyrrolidinone and pyrrolidine-thione compounds (US2003/0149081, US Patent
 6,727,275 & US Patent 6,936,633); mercaptoimidazole compounds
 15 (US2007/0244138); substituted dipiperidine compounds (US2007/0197590);
 phenylamino substituted quaternary salt compounds (US2006/0293379); bicyclic
 and bridged nitrogen heterocycles (US2006/0074121); 3-
 aminocyclopentanecarboxamide compounds (US2006/0020133); 3-(4-
 heteroaryl)cyclohexylamino)cyclopentanecarboxamide compounds
 20 (US2005/0267146); triazolo compounds (US Patent 6,492,364); heteroaryl
 sulfonamide compounds (US2006/0173019); aryl sulfonamides (US Patent
 6,939,885); bis-aryl sulfonamides (US Patent 7,227,035 & US2004/0167113);
 substituted benzamide compounds (US Patent 6,821,964); substituted diazepam
 compounds (US2007/0249589); Triazaspiro[5.5]undecane derivatives
 25 (US2005/267114); substituted piperidinecarboxamide compounds
 (US2003/0114443 & US6,562,978); benzazepine derivatives (US2004/0235822 &
 US Patent 7,262,185);

[00192] In selected cases the chemokine antagonist co-formulated or co-
 administered with the CCR2 antibodies is SELZENTRY™ (Maraviroc), which is
 30 chemically described as 4,4-difluoro-*N*-{(1*S*)-3-[*exo*-3-(3-isopropyl-5-methyl-4*H*-
 1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-
 phenylpropyl}cyclohexanecarboxamide:



[00193] In certain specific cases, the therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is a CB-1
 5 receptor antagonist. As used herein, the term "CB-1 receptor" refers to a G-protein coupled type 1 cannabinoid receptor. The term "antagonist" includes both full antagonists and partial antagonists, as well as inverse agonists. The CB-1 receptor antagonist may be selective to the CB-1 receptor. "CB-1 receptor selective" means that the compound has little or no activity to antagonize the
 10 cannabinoid-2 receptor (CB-2). The CB-1 antagonist may be at least about 10 fold selective for the CB-1 receptor in comparison to the CB-2 receptor. For example, the inhibitory concentration (IC₅₀) for antagonizing the CB-1 receptor is about 10 or more times lower than the IC₅₀ for antagonizing the CB-2 receptor. Suitable CB-1 receptor antagonists include compounds disclosed in U.S. Patent Nos.
 15 5,462,960; 5,596,106; 5,624,941 ; 5,747,524; 6,017,919; 6,028,084; 6,432,984; 6,476,060; 6,479,479; 6,518,264; and 6,566,356; U.S. Patent Publication Nos. 2003/0114495; 2004/0077650; 2004/0092520; 2004/0122074; 2004/0157838; 2004/0157839; 2004/0214837; 2004/0214838; 2004/0214855; 2004/0214856; 2004/0058820; 2004/0235926; 2004/0259887; 2005/0080087; 2005/0026983 and
 20 2005/0101592; PCT Patent Publication Nos. WO 03/075660; WO 02/076949; WO 01/029007; WO 04/048317; WO 04/058145; WO 04/029204; WO 04/012671 ; WO 03/087037; WO 03/086288; WO 03/082191 ; WO 03/082190; WO 03/063781 ; WO 04/012671 ; WO 04/013120; WO 05/020988; WO 05/039550; WO 05/044785; WO 05/044822; and WO 05/049615; PCT Patent Application Serial
 25 Nos. PCT/IB2004/004050 filed on December 6, 2004; PCT/IB2004/004017 filed

on December 6, 2004; PCT/IB2004/004023 filed on December 6, 2004; and PCT/IB2004/004019 filed on December 6, 2004; and U.S. Provisional Application Nos. 60/523937 filed on November 21, 2003; 60/529908 filed on December 16, 2003; 60/529909 filed on December 16, 2003; 60/529910 filed on December 16, 2003; 60/530012 filed on December 16, 2003; and 60/564648 filed on April 21, 2004. All of the above patents and patent applications are incorporated herein by reference. Preferred CB-1 receptor antagonists for use in the methods include: rimonabant (SR141716A also known under the tradename Acomplia™) is available from Sanofi-Synthelabo or can be prepared as described in U.S. Patent No. 5,624,941; *N*-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) is available from Tocris™, Ellisville, MO; [5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-ethyl-*N*-(1-piperidinyl)-1H-pyrazole-3-carboxamide] (SR147778) which can be prepared as described in US Patent No. 6,645,985; *N*-(piperidin-1-yl)-4,5-diphenyl-1-methylimidazole-2-carboxamide, *N*-(piperidin-1-yl)-4-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-1-methylimidazole-2-carboxamide, *N*-(piperidin-1-yl)-4,5-di-(4-methylphenyl)-1-methylimidazole-2-carboxamide, *N*-cyclohexyl-4,5-di-(4-methylphenyl)-1-methylimidazole-2-carboxamide, *N*-(cyclohexyl)-4-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-1-methylimidazole-2-carboxamide, and *N*-(phenyl)-4-(2,4-dichlorophenyl)-5-(4-chlorophenyl)-1-methylimidazole-2-carboxamide which can be prepared as described in PCT Patent Publication No. WO 03/075660; the hydrochloride, mesylate and besylate salt of 1-[9-(4-chlorophenyl)-8-(2-chlorophenyl)-9H-purin-6-yl]-4-ethylamino-piperidine-4-carboxylic acid which can be prepared as described in U.S. Patent Publication No. 2004/0092520; 1-[7-(2-chlorophenyl)-8-(4-chlorophenyl)-2-methyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]-3-ethylamino-azetidine-3-carboxylic acid amide and 1-[7-(2-chlorophenyl)-8-(4-chlorophenyl)-2-methyl-pyrazolo[1,5-a][1,3,5]triazin-4-yl]-3-methylamino-azetidine-3-carboxylic acid amide which can be prepared as described in U.S. Patent Publication No. 2004/0157839; 3-(4-chlorophenyl)-2-(2-chlorophenyl)-6-(2,2-difluoro-propyl)-2,4,5,6-tetrahydro-pyrazolo[3,4-c]pyridin-7-one, 2-(2-chlorophenyl)-3-(4-ethylphenyl)-5-(2,2,2-trifluoro-ethyl)-4,5-dihydro-2H-pyrrolo[3,4-c]pyrazol-6-one, and 2-(2-chlorophenyl)-3-(4-isopropylphenyl)-5-(2,2,2-trifluoro-ethyl)-4,5-dihydro-2H-pyrrolo[3,4-c]pyrazol-6-one which can be prepared as

described in U.S. Patent Publication No. 2004/0214855; 3-(4-chloro-phenyl)-2-(2-chloro-phenyl)-7-(2,2-difluoro-propyl)-6,7-dihydro-2H,5H-4-oxa-1,2,7-triazazulen-8-one which can be prepared as described in U.S. Patent Publication No. 2005/0101592; 2-(2-chloro-phenyl)-6-(2,2,2-trifluoro-ethyl)-3-(4-trifluoromethyl-phenyl)-2,6-dihydro-pyrazolo[4,3-d]pyrimidin-7-one which can be prepared as described in U.S. Patent Publication No. 2004/0214838; (S)-4-chloro-N-{{3-(4-chloro-phenyl)-4-phenyl-4,5-dihydro-pyrazol-1-yl}-methylamino-methylene}-benzenesulfonamide (SLV-319) and (S)-N-{{3-(4-chloro-phenyl)-4-phenyl-4,5-dihydro-pyrazol-1-yl}-methylamino-methylene}-4-trifluoromethyl-
 10 benzenesulfonamide (SLV-326) which can be prepared as described in PCT Patent Publication No. WO 02/076949; N-piperidino-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-ethylpyrazole-3-carboxamide which can be prepared as described in U.S. Patent No. 6,432,984; 1-[bis-(4-chloro-phenyl)-methyl]-3-[(3,5-difluoro-phenyl)-methanesulfonyl-methylene]-azetidine which can be prepared as described in U.S. Patent No. 6,518,264; 2-(5-(trifluoromethyl)pyridin-2-yloxy)-N-(4-(4-chlorophenyl)-3-(3-cyanophenyl)butan-2-yl)-2-methylpropanamide which can be prepared as described in PCT Patent Publication No. WO 04/048317; 4-{{6-methoxy-2-(4-methoxyphenyl)-1-benzofuran-3-yl}carbonyl}benzonitrile (LY-320135) which can be prepared as described in U.S. Patent No. 5,747,524; 1-[2-(2,4-dichlorophenyl)-2-(4-fluorophenyl)-benzo[1,3]dioxole-5-sulfonyl]-piperidine which can be prepared as described in WO 04/013120; and [3-amino-5-(4-chlorophenyl)-6-(2,4-dichlorophenyl)-furo[2,3-b]pyridin-2-yl]-phenyl-methanone which can be prepared as described in WO 04/012671.

[00194] In certain specific cases, the therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is an angiogenic factor. Angiogenic factors include, but are not limited to, basic fibroblast growth factor, acidic fibroblast growth factor, vascular endothelial growth factor, angiogenin, transforming growth factor α and β tumor necrosis factor, angiopoietin, platelet-derived growth factor, placental growth factor, hepatocyte growth factor, and proliferin.

[00195] In certain specific cases, the therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is an thrombolytic agent. Thrombolytic agents include, but are not limited to, urokinase

plasminogen activator, urokinase, streptokinase, inhibitors of α 2-plasmin inhibitor, and inhibitors of plasminogen activator inhibitor-1, angiotensin converting enzyme (ACE) inhibitors, spironolactone, tissue plasminogen activator (tPA), an inhibitor of interleukin 1 β -converting enzyme, anti-thrombin III, and the like.

- 5 **[00196]** In certain specific cases, the therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is an anti-obesity agent. Anti-obesity agents include, but are not limited to, an apo-B/MTP inhibitor, a 11 β -hydroxy steroid dehydrogenase-1 inhibitor, peptide YY₃₋₃₆ or an analog thereof, a MCR-4 agonist, a CCK-A agonist, a monoamine reuptake
- 10 inhibitor, a sympathomimetic agent, a β ₃ adrenergic receptor agonist, a dopamine agonist, a melanocyte-stimulating hormone receptor analog, a 5-HT_{2c} receptor agonist, a melanin concentrating hormone antagonist, leptin, a leptin analog, a leptin receptor agonist, a galanin antagonist, a lipase inhibitor, a bombesin agonist, a neuropeptide-Y receptor antagonist, a thyromimetic agent,
- 15 dehydroepiandrosterone or analog thereof, a glucocorticoid receptor antagonist, an orexin receptor antagonist, a glucagon-like peptide-1 receptor agonist, a ciliary neurotrophic factor, a human agouti-related protein antagonist, a ghrelin receptor antagonist, a histamine 3 receptor antagonist or inverse agonist, and a neuromedin U receptor agonist.
- 20 **[00197]** In certain specific cases, the therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is a agent which inhibits the recruitment and/or adhesion of neutrophils and/or mononuclear cells to a site of vascular injury. Such therapeutic agents can, for example, inhibit the activity (e.g., binding activity, signaling activity) of a cell surface molecule
- 25 through which cellular adhesion, chemotaxis and/or homing are mediated. For example, antagonists of cellular adhesion molecules (e.g., integrins (e.g., β 1, β 2, β 3, β 4, β 5, β 6, β 7, β 8 integrins), selectins (e.g., E-selectin, P-selectin, L-selectin), cadherins (e.g., E-, P-, N-cadherins) and immunoglobulin superfamily adhesion molecules (e.g., LFA-2, LFA-3, CD44)) and antagonists of cytokine receptors
- 30 (e.g., antagonists of chemokine receptor function) can be co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody. In addition, agents which bind to ligands of cellular adhesion molecules or cytokines or chemokines and inhibit the binding of ligand to receptors expressed on neutrophils and/or

mononuclear cells can also be co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody.

[00198] In certain specific cases, the therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is an cardiac therapeutic agent. Exemplary therapeutic agents intended to treat cardiac disorders include, but are not limited to, growth factors, angiogenic agents, calcium channel blockers, antihypertensive agents, inotropic agents, antiatherogenic agents, anti-coagulants, β -blockers, anti-arrhythmia agents, cardiac glycosides, antiinflammatory agents, antibiotics, antiviral agents, antifungal agents and agents that inhibit protozoan infections, and antineoplastic agents.

[00199] In certain specific cases, the cardiac therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is a calcium channel blocker. Calcium channel blockers include, but are not limited to, dihydropyridines such as nifedipine, nicardipine, nimodipine, and the like; benzothiazepines such as diltiazem; phenylalkylamines such as verapamil; diarylaminopropylamine ethers such as bepridil; and benzimidole-substituted tetralines such as mibefradil.

[00200] In certain specific cases, the cardiac therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is an antihypertensive agent. Antihypertensive agents include, but are not limited to, diuretics, including thiazides such as hydrochlorothiazide, furosemide, spironolactone, triamterene, and amiloride; antiadrenergic agents, including clonidine, guanabenz, guanfacine, methyldopa, trimethaphan, reserpine, guanethidine, guanadrel, phentolamine, phenoxybenzamine, prazosin, terazosin, doxazosin, propranolol, methoprolol, nadolol, atenolol, timolol, betaxolol, carteolol, pindolol, acebutolol, labetalol; vasodilators, including hydralazine, minoxidil, diazoxide, nitroprusside; and angiotensin converting enzyme inhibitors, including captopril, benazepril, enalapril, enalaprilat, fosinopril, lisinopril, quinapril, ramipril; angiotensin receptor antagonists, such as losartan; and calcium channel antagonists, including nifedipine, amlodipine, felodipine XL, isradipine, nicardipine, benzothiazepines (e.g., diltiazem), and phenylalkylamines (e.g. verapamil).

5 **[00201]** In certain specific cases, the cardiac therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is an anti-coagulant. Anti-coagulants include, but are not limited to, heparin, warfarin, hirudin, tick anti-coagulant peptide, low molecular weight heparins such as enoxaparin, dalteparin, and ardeparin, ticlopidine, danaparoid, argatroban, abciximab and tirofiban.

10 **[00202]** In certain specific cases, the cardiac therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is an antiarrhythmic agent. Antiarrhythmic agents include, but are not limited to, sodium channel blockers (e.g., lidocaine, procainamide, encainide, flecainide, and the like), beta adrenergic blockers (e.g., propranolol), prolongers of the action potential duration (e.g., amiodarone), and calcium channel blockers (e.g., verapamil, diltiazem, nickel chloride, and the like). Delivery of cardiac depressants (e.g., lidocaine), cardiac stimulants (e.g., isoproterenol, dopamine,

15 norepinephrine, etc.), and combinations of multiple cardiac agents (e.g., digoxin/quinidine to treat atrial fibrillation) is also of interest.

20 **[00203]** In certain specific cases, the cardiac therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is an agent for treating congestive heart failure. Agents for treating congestive heart failure include, but are not limited to, a cardiac glycoside, inotropic agents, a extracellular loop diuretic, a thiazide diuretic, a potassium ion sparing diuretic, an angiotensin converting enzyme inhibitor, an angiotensin receptor antagonist, a nitro vasodilator, a phosphodiesterase inhibitor, a direct vasodilator, an α 1-adrenergic receptor antagonist, a calcium channel blocker, and a

25 sympathomimetic agent.

30 **[00204]** In certain specific cases, the cardiac therapeutic agent(s) that is co-formulated with and/or co-administered with an inhibitory anti-CCR2 antibody is an agent suitable for treating cardiomyopathies, such as but not limited to, dopamine, epinephrine, norepinephrine, and phenylephrine.

[00205] Also included are compositions for inhibiting viral infection, and in particular HIV-infection, in a mammal comprising an amount of an antibody in combination with an amount of an antiviral agent, wherein the amounts of the anti-CCR2 antibody and of antiviral agent are together effective in inhibiting viral

replication, viral infection of new cells or viral loads. Many antiviral agents are presently known in the art, including nucleoside analogues (e.g., AZT, 3TC, and ddI), protease inhibitors and chemokine receptor antagonists may inhibit HIV infection but not viral infections.

- 5 **[00206]** In another aspect, the anti-CCR2 antibody or fragment thereof may be co-administered with other therapeutic agents, such as antiinflammatory drugs or molecules, to a patient who has a inflammatory disorder, such as arthritis, atherosclerosis or multiple sclerosis. In one aspect, methods for the treatment of the inflammatory disorder in a mammal comprising administering to said mammal
- 10 a therapeutically effective amount of a compound in combination with an antiinflammatory agent are provided. Antiinflammatory agents include, but are not limited to, any known nonsteroidal antiinflammatory agent such as, salicylic acid derivatives (aspirin), para-aminophenol derivatives (acetaminophen), indole and indene acetic acids (indomethacin), heteroaryl acetic acids (ketorolac),
- 15 arylpropionic acids (ibuprofen), anthranilic acids (mefenamic acid), enolic acids (oxicams) and alkanones (nabumetone) and any known steroidal antiinflammatory agent which include corticosteroids and biologically active synthetic analogs with respect to their relative glucocorticoid (metabolic) and mineralocorticoid (electrolyte-regulating) activities. In another case, the anti-CCR2 antibody is
- 20 administered in combination with a non-steroidal anti-inflammatory drug such as aspirin (Bayer, Bufferin), ibuprofen (Motrin, Advil), naproxen sodium (Aleve), ketoprofen (Orudis KT), indomethacin (Indocin), etodolac (Lodine), diclofenac sodium (Voltaren), rofecoxib (Vioxx), celecoxib (Celebrex), nabumetone (Relafen) or in combination with a steroid such as prednisone, prednisolone,
- 25 dexamethasone, beclomethasone, budesonide, fluticasone or triamcinolone.
- [00207]** Additionally, other drugs used in the therapy of inflammation include but are not limited to antagonists such as all histamine and bradykinin receptor antagonists, leukotriene and prostaglandin receptor antagonists, and platelet activating factor receptor antagonists. In still another case, the antibody or
- 30 combination therapy is administered along with radiotherapy, chemotherapy, photodynamic therapy, surgery or other immunotherapy treatment *i.e.* therapy targeting the immune system. In yet still another case, the antibody will be administered with another antibody. For example, the anti-CCR2 antibody may

be administered with an antibody or other agent that is known to inhibit inflammation, e.g., an antibody or agent that inhibits alpha-4 integrin (US2004/0009169) or IL-8 receptor (US2004/0037830). Additional antibodies that may be coadministered with the anti-CCR2 antibodies are described in U.S.

- 5 Patent Nos. 6,696,50; 6,406,865; 6,352,832; and 6,084,075, the contents of which are incorporated by reference herein.

[00208] The compositions may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antigen-binding portion. A "therapeutically effective amount" refers to an amount effective, at dosages and
10 for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which
15 any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically
20 effective amount may be less than the therapeutically effective amount.

[00209] Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus can be administered, several divided doses can be administered over time or the dose can be proportionally reduced or increased as indicated by the
25 exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to
30 produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms are dictated by and directly dependent on (a) the unique characteristics of the anti-CCR2 antibody or portion thereof and the particular therapeutic or prophylactic effect to

be achieved, and (b) the limitations inherent in the art of compounding such an antibody for the treatment of sensitivity in individuals.

[00210] An exemplary, non-limiting range for a therapeutically or prophylactically-effective amount of an antibody or antibody portion is 0.025 to 50 mg/kg, 0.1 to 50 mg/kg, 0.1-25, 0.1 to 10 or 0.1 to 3 mg/kg. In one case, the antibody is administered in a formulation as a sterile aqueous solution having a pH that ranges from about 5.0 to about 6.5 and comprising from about 1 mg/ml to about 200 mg/ml of antibody, from about 1 millimolar to about 100 millimolar of histidine buffer, from about 0.01 mg/ml to about 10 mg/ml of polysorbate 80 or polysorbate 20, from about 100 millimolar to about 400 millimolar of a non-reducing sugar selected from but not limited to trehalose or sucrose, from about 0.01 millimolar to about 1.0 millimolar of disodium EDTA dihydrate and optionally comprise a pharmaceutically acceptable antioxidant in addition to a chelating agent. Suitable antioxidants include, but are not limited to, methionine, sodium thiosulfate, catalase, and platinum. For example, the composition may contain methionine in a concentration that ranges from 1 mM to about 100 mM, and in particular, is about 27 mM. In some cases, a formulation contains 5 mg/ml of antibody in a buffer of 20mM sodium citrate, pH 5.5, 140mM NaCl, and 0.2 mg/ml polysorbate 80. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

[00211] Another aspect provides kits comprising an anti-CCR2, or antigen-binding portion, or a composition comprising such an antibody or antigen-binding fragment. A kit may include, in addition to the antibody or composition, diagnostic or therapeutic agents. A kit can also include instructions for use in a diagnostic or therapeutic method, as well as packaging material such as, but not limited to, ice, dry ice, STYROFOAM™, foam, plastic, cellophane, shrink wrap, bubble wrap, cardboard and starch peanuts. In one case, the kit includes the antibody or a composition comprising it and a diagnostic agent that can be used in a method

described herein. In still another case, the kit includes the antibody or a composition comprising it and one or more therapeutic agents that can be used in a method described herein.

- 5 [00212] Compositions and kits for inhibiting cancer in a mammal comprising an amount of an antibody in combination with an amount of a chemotherapeutic agent, wherein the amounts of the compound, salt, solvate, or prodrug, and of the chemotherapeutic agent are together effective in inhibiting abnormal cell growth are provided. Many chemotherapeutic agents are presently known in the art. In some cases, the chemotherapeutic agent is selected from the group consisting of
- 10 mitotic inhibitors, alkylating agents, anti-metabolites, intercalating antibiotics, chemokine inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, anti-hormones, e.g., anti-androgens, and anti-angiogenesis agents.

Diagnostic Methods of Use

- 15 [00213] In another aspect, diagnostic methods are provided. The anti-CCR2 antibodies can be used to detect CCR2 in a biological sample *in vitro* or *in vivo*. In one case, a method is provided for diagnosing the presence or location of an CCR2-expressing cells in a subject in need thereof, comprising the steps of injecting the antibody into the subject, determining the expression of CCR2 in the
- 20 subject by localizing where the antibody has bound, comparing the expression in the subject with that of a normal reference subject or standard, and diagnosing the presence or location of the cells. The anti-CCR2 antibodies may also be used as a marker for inflammation and/or for the infiltration of immune cells, such as monocytes, into a tissue.
- 25 [00214] The anti-CCR2 antibodies can be used in a conventional immunoassay, including, without limitation, an ELISA, an RIA, flow cytometry, tissue immunohistochemistry, Western blot or immunoprecipitation. The anti-CCR2 antibodies may be used to detect CCR2 from humans. In another case, the anti-CCR2 antibodies may be used to detect CCR2 from cynomolgus monkeys or
- 30 rhesus monkeys. In another case, the anti-CCR2 antibodies may be used to detect CCR2 from rodents, such as mice and rats.

[00215] Also provided is a method for detecting CCR2 in a biological sample comprising contacting the biological sample with an anti-CCR2 antibody and detecting the bound antibody. In one case, the anti-CCR2 antibody is directly labeled with a detectable label. In another case, the anti-CCR2 antibody (the first antibody) is unlabeled and a second antibody or other molecule that can bind the anti-CCR2 antibody is labeled. As is well known to one of skill in the art, a second antibody is chosen that is able to specifically bind the particular species and class of the first antibody. For example, if the anti-CCR2 antibody is a human IgG, then the secondary antibody could be an anti-human-IgG. Other molecules that can bind to antibodies include, without limitation, Protein A and Protein G, both of which are available commercially, e.g., from Pierce Chemical Co.

[00216] Suitable labels for the antibody or secondary antibody have been disclosed supra, and include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β -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; and examples of suitable radioactive material include ^{125}I , ^{131}I , ^{35}S or ^3H .

[00217] In other cases, CCR2 can be assayed in a biological sample by a competition immunoassay utilizing CCR2 standards labeled with a detectable substance and an unlabeled anti-CCR2 antibody. In this assay, the biological sample, the labeled CCR2 standards and the anti-CCR2 antibody are combined and the amount of labeled CCR2 standard bound to the unlabeled antibody is determined. The amount of CCR2 in the biological sample is inversely proportional to the amount of labeled CCR2 standard bound to the anti-CCR2 antibody.

[00218] One can use the immunoassays disclosed above for a number of purposes. For example, the anti-CCR2 antibodies can be used to detect CCR2 in cultured cells. In one case, the anti-CCR2 antibodies are used to determine the amount of CCR2 on the surface of cells that have been treated with various

compounds. This method can be used to identify compounds that modulate CCR2 protein levels. According to this method, one sample of cells is treated with a test compound for a period of time while another sample is left untreated. If the total CCR2 expression is to be measured, the cells are lysed and the total CCR2 expression is measured using one of the immunoassays described herein. The total CCR2 expression in the treated versus the untreated cells is compared to determine the effect of the test compound.

[00219] A preferred immunoassay for measuring total CCR2 expression is flow cytometry or immunohistochemistry. If the cell surface CCR2 expression is to be measured, the cells are not lysed, and the cell surface levels of CCR2 are measured using one of the immunoassays described herein. A preferred immunoassay for determining cell surface levels of CCR2 includes the steps of labeling the cell surface proteins with a detectable label, such as biotin or ¹²⁵I, immunoprecipitating the CCR2 with an anti-CCR2 antibody and then detecting the labeled CCR2.

[00220] Another immunoassay for determining the localization of CCR2, e.g., cell surface levels, is by using immunohistochemistry. An immunoassay to detect cell surface levels of CCR2 includes binding of an anti-CCR2 antibody labeled with an appropriate fluorophore, such as fluorescein or phycoerythrin, and detecting the primary antibody using flow cytometry. In another case, the anti-CCR2 antibody is unlabeled and a second antibody or other molecule that can bind the anti-CCR2 antibody is labeled. Methods such as ELISA, RIA, flow cytometry, Western blot, immunohistochemistry, cell surface labeling of integral membrane proteins and immunoprecipitation are well known in the art. See, e.g., Harlow and Lane, supra. In addition, the immunoassays can be scaled up for high throughput screening in order to test a large number of compounds for either activation or inhibition of CCR2.

[00221] The anti-CCR2 antibodies also can be used to determine the levels of CCR2 in a tissue or in cells derived from the tissue. In some cases, the tissue is a diseased tissue. In some cases, the tissue is a tissue biopsy. In some cases of the method, a tissue or a biopsy thereof is excised from a patient. The tissue or biopsy is then used in an immunoassay to determine, e.g., total CCR2 expression, cell surface levels of CCR2 or localization of CCR2 by the methods discussed

above. Such methods can be used to determine whether a tissue expresses high levels of CCR2, which could be indicative that the tissue is a target for treatment with anti-CCR2 antibody.

5 [00222] The antibodies also can be used *in vivo* to identify tissues and organs that express CCR2. In some cases, the anti-CCR2 antibodies are used to identify CCR2-expressing cells.

[00223] The method comprises the steps of administering a detectably labeled anti-CCR2 antibody or a composition comprising them to a patient in need of such a diagnostic test and subjecting the patient to imaging analysis to determine the
10 location of the CCR2-expressing tissues. Imaging analysis is well known in the medical art, and includes, without limitation, X-ray analysis, magnetic resonance imaging (MRI) or computed tomography (CT). The antibody can be labeled with any agent suitable for *in vivo* imaging, for example a contrast agent, such as
15 barium, which can be used for X-ray analysis, or a magnetic contrast agent, such as a gadolinium chelate, which can be used for MRI or CT. Other labeling agents include, without limitation, radioisotopes, such as ⁹⁹Tc. In another case, the anti-CCR2 antibody will be unlabeled and will be imaged by administering a second antibody or other molecule that is detectable and that can bind the anti-CCR2 antibody. In another case, a biopsy is obtained from the patient to determine
20 whether the tissue of interest expresses CCR2.

[00224] In some cases, the detectably labeled anti-CCR2 comprises a fluorophore. In certain cases, the fluorophore is selected from the group consisting of a near-infrared fluorescent dye, dinitrophenyl, fluorescein and derivatives thereof, rhodamine, derivatives of rhodamine, phycoerythrin,
25 phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine, TEXAS RED™, RHODAMINE GREEN™, OREGON GREEN™, CASCADE BLUE™, phycoerythrin, CY3™, CY5™, CY2™, CY7™, coumarin, infrared 40, MR 200, IRD 40, ALEXA FLUOR™, Tetramethylrhodamine, PACIFIC BLUE™, SYBR™, and BODIPY™. In another case, the fluorophore includes one of the following
30 compounds with their emission maxima indicated in nm in brackets, CY2™ (506), GFP (Red Shifted) (507), YO-PRO®-1 (509), YOYO®-1 (509), Calcein (517), FITC (518), FLUORX® (519), ALEXA® (520), Rhodamine 110 (520), 5-FAM (522), OREGON GREEN® 500 (522), OREGON GREEN® 488 (524),

RIBOGREEN® (525), RHODAMINE GREEN® (527), Rhodamine 123 (529),
 MAGNESIUM GREEN® (531), CALCIUM GREEN® (533), TO-PRO®-1 (533),
 TOTO®-1 (533), JOE (548), BODIPY® 530/550 (550), DiI (565), BODIPY® (568),
 BODIPY® 558/568 (568), BODIPY® 564/570 (570), CY3® (570), ALEXA® 546
 5 (570), TRITC (572), MAGNESIUM ORANGE® (575), Phycoerythrin R&B (575),
 Rhodamine Phalloidin (575), CALCIUM ORANGE® (576), Pyronin Y (580),
 Rhodamine B (580), TAMRA (582), RHODAMINE RED® (590), CY3.5® (596),
 ROX (608), CALCIUM CRIMSON™ (615), ALEXA® 594 (615), TEXAS RED™
 (615), Nile Red (628), YO-PRO®-3 (631), YOYO®-3 (631), R-phycocyanin (642),
 10 C-Phycocyanin (648), TO-PRO®-3 (660), TOTO®-3 (660), DiD DiI(5) (665),
 CY5™ (670), Thiadcarbocyanine (671) and Cy5.5™ (694).

Therapeutic Methods of Use

[00225] In another aspect, provided are methods for inhibiting CCR2 activity by
 administering an anti-CCR2 antibody to a patient in need thereof. Any of the
 15 types of antibodies described herein may be used therapeutically. In various
 cases, the anti-CCR2 antibody is a human, chimeric or humanized antibody. In
 some cases, the antibody, or antigen-binding portion thereof, binds to the first
 and/or second extracellular loop of CCR2. The antibody, or antigen-binding
 portion thereof, preferably does not bind to the third extracellular loop or to the N-
 20 terminal domain of CCR2.

[00226] In still another case, the CCR2 is human and the patient is a human
 patient. Alternatively, the patient may be a mammal that expresses a CCR2 that
 the anti-CCR2 antibody cross-reacts with. The antibody may be administered to a
 non-human mammal expressing CCR2 with which the antibody cross-reacts for
 25 veterinary purposes or as an animal model of human disease. Such animal
 models may be useful for evaluating the therapeutic efficacy of antibodies.

[00227] In one aspect, methods are provided for treating, aiding in the treatment,
 preventing or aiding in the prevention of, a CCR2-mediated disorder in a subject
 by administering to the subject a therapeutically-effective amount of an anti-CCR2
 30 antibody. As used herein, the term "a CCR2-mediated disorder" is intended to
 include diseases and other disorders in which the presence of high levels of
 CCR2 expression or activity in a subject suffering from the disorder have been

shown to be, or are suspected of being, either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Such disorders may be evidenced, for example, by an increase in the levels of CCR2 on the cell surface in the affected cells or tissues of a subject suffering from the disorder, or by an increase in a CCR2-mediated activity in a cell type, such as in a basophil, monocyte or lymphocyte, that contributes to the pathology of the disorder or that contributes to the worsening of the disorder or by an increase in the level of CCR2 ligands, such as MCP-1, at an inflammatory site. The increase in CCR2 expression may be detected, for example, using an anti-CCR2 antibody.

An increase in CCR2 activity may be detected by an increase in activation of G-protein, F-actin polymerization or increased chemotaxis of CCR2-expressing cells, such as chemotaxis in response to MCP-1 or other CCR2 ligands.

[00228] In one aspect, the CCR2-mediated disorder is characterized by fibrosis. The term "fibrosis" as used herein refers to a pathological condition characterized by excessive deposition and metabolism of fibrotic material (e.g., extracellular matrix) in response to tissue damage. In many cases, fibrosis represents a normal repair process (i.e., wound healing) gone awry due to chronic or excessive tissue insult leading to fibroblast or stellate cell activation and proliferation and collagen accumulation. Fibrosis conditions include fibroproliferative disorders that are associated with vascular diseases, such as cardiac disease, cerebral disease, and peripheral vascular disease, as well as all the main tissues and organ systems such as the eye, skin, kidney, lung, gut and liver (Wynn, *Nature Reviews* 4:583-594 (2004); Bataller, R and Brenner, D., *J. Clin. Invest.* 115:209-218 (2005)). Other sources are chemotherapeutic drugs, radiation-induced fibrosis, and injuries and burns. While fibrosis conditions cover a wide group of pathologies, it is believed that for most of these conditions, the general mechanisms leading to fibrotic tissue accumulation have many elements in common. Often the condition is initiated in response to an influx of inflammatory cells and perpetuated by the subsequent cytokine signaling pathways between the infiltrating cells (e.g., macrophages, T cells) and resident cells within the tissue (e.g., stellate, myofibroblast or Kupffer cells). For example, MCP-1 has been shown to play a role in several diseases of the lung (Rose CE Jr, Sung SS, Fu SM. *Microcirculation* 10:273-288 (2003)) and CCR2 deficient mice are protected

from development of lung fibrosis (Moore BB, et al. Protection from Pulmonary Fibrosis in the Absence of CCR2 Signaling. *J. Immunol.* 167: 4368-4377 (2001)), suggesting a key role of this receptor in the lung. Similarly, pericytes are a key fibrogenic cell type involved in the development of scleroderma and PDGF

5 receptor tyrosine kinase inhibitors (RTKI) have been shown to slow the proliferation of pericytes and suppress skin lesions in patients with this progressive disease. In the kidney, leukocyte infiltration plays a major role in mediating tubulointerstitial inflammation and fibrosis in chronic kidney disease. Vielhauer and colleagues have established that in a mouse model of obstructive

10 nephropathy, expression of CCR2 and CCR5 on accumulating macrophages and CD3⁺ lymphocytes correlates with progressive fibrosis at sites of tissue damage (Vielhauer V, et al. *J. Am. Soc. Nephrol.* 12:1173-1187 (2001)).

[00229] As used herein the term "fibrosis" is also used synonymously with "fibroblast accumulation and collagen deposition". Fibroblasts are connective

15 tissue cells, which are dispersed in connective tissue throughout the body. Fibroblasts secrete a nonrigid extracellular matrix containing type I and/or type III collagen. In response to an injury to a tissue, nearby fibroblasts or stellate cells migrate into the wound, proliferate, and produce large amounts of collagenous extracellular matrix. Collagen is a fibrous protein rich in glycine and proline that is

20 a major component of the extracellular matrix and connective tissue, cartilage, and bone. Collagen molecules are triple-stranded helical structures called α -chains, which are wound around each other in a ropelike helix. Collagen exists in several forms or types; of these, type I, the most common, is found in skin, tendon, and bone; and type III is found in skin, blood vessels, and internal organs.

25 Exemplary fibrosis conditions include, but are not limited to, lung diseases associated with fibrosis, e.g., idiopathic pulmonary fibrosis, radiation induced fibrosis, chronic obstructive pulmonary disease (COPD), scleroderma, bleomycin induced pulmonary fibrosis, chronic asthma, silicosis, asbestos induced pulmonary fibrosis, acute lung injury and acute respiratory distress (including

30 bacterial pneumonia induced, trauma induced, viral pneumonia induced, ventilator induced, non-pulmonary sepsis induced, and aspiration induced); chronic nephropathies associated with injury/fibrosis (kidney fibrosis), e.g., lupus, diabetes, scleroderma, glomerular nephritis, focal segmental glomerular sclerosis,

IgA nephropathy, hypertension, allograft, Lupus, and Alport; gut fibrosis, e.g., scleroderma, and radiation induced gut fibrosis; liver fibrosis, e.g., cirrhosis, alcohol induced liver fibrosis, nonalcoholic steatohepatitis (NASH), biliary duct injury, primary biliary cirrhosis, infection or viral induced liver fibrosis (e.g. chronic
5 HCV infection), and autoimmune hepatitis; head and neck fibrosis, e.g., radiation induced; corneal scarring, e.g., LASIX™, corneal transplant, and trabeculectomy; hypertrophic scarring and keloids, e.g., burn induced and surgical; and other fibrotic diseases, e.g., sarcoidosis, scleroderma, spinal cord injury/fibrosis, myelofibrosis, vascular restenosis, atherosclerosis, Wegener's granulomatosis,
10 mixed connective tissue disease, and Peyronie's disease.

[00230] In one aspect, the CCR2-mediated disorder is characterized by pathological inflammation. The term "pathological inflammation" as used herein refers to an inappropriate and/or chronic inflammation associated with disorders including, but not limited to, asthma, atherosclerosis, AIDS dementia, diabetes,
15 inflammatory bowel disease, rheumatoid arthritis, transplant rejection, graft versus host disease, multiple sclerosis (especially to inhibit further demyelination), tumors, tumor metastasis, nephritis, atopic dermatitis, psoriasis, myocardial ischemia and chronic prostatitis, obesity, metabolic syndrome. Such inflammation is characterized by a heightened response of inflammatory cells, including
20 infiltrating leukocytes. Over time, such pathological inflammation often results in damage to tissue in the region of inappropriate inflammation. Accordingly, provided are methods of treating a subject having pathological inflammation comprising administering to the subject a therapeutically effective amount of an antibody or antigen-binding portion thereof which binds to and inhibits CCR2.

25 **[00231]** The antibody or an antigen-binding fragments thereof, can also be used to treat disorders in which activation of the CCR2 by binding of chemokines, including MCP-1, MCP-2, MCP-3 and MCP-4, is implicated.

[00232] The antagonistic anti-CCR2 antibodies or antigen-binding fragments thereof (e.g., 4.40 and/or 4.9 or antigen-binding fragments thereof) can be used to
30 treat inflammatory or allergic diseases and conditions, including respiratory allergic diseases such as asthma, allergic rhinitis, hypersensitivity lung diseases, hypersensitivity pneumonitis, interstitial lung diseases (ILD) (e.g., idiopathic pulmonary fibrosis, or ILD associated with rheumatoid arthritis, systemic lupus

- erythematosus, ankylosing spondylitis, systemic sclerosis, Sjogren's syndrome, polymyositis or dermatomyositis), chronic obstructive pulmonary disease, anaphylaxis or hypersensitivity responses, drug allergies (e.g., to penicillin, cephalosporins), insect sting allergies, inflammatory bowel diseases, such as
- 5 Crohn's disease and ulcerative colitis, spondyloarthropathies, scleroderma, psoriasis and inflammatory dermatoses such as dermatitis, eczema, atopic dermatitis, allergic contact dermatitis, urticaria, vasculitis (e.g., necrotizing, cutaneous, and hypersensitivity vasculitis).
- [00233]** The antagonistic anti-CCR2 antibodies or antigen-binding fragments thereof (e.g., 4.40 and/or 4.9 or antigen-binding fragments thereof) can be used to
- 10 treat autoimmune diseases. Examples of autoimmune disorders include, but are not limited to, Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia
- 15 Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disease.
- 20 **[00234]** In certain cases, the autoimmune disease treated with the antagonistic anti-CCR2 antibodies is Rheumatoid Arthritis (RA). A TH1 disorder, RA is a common human autoimmune disease with a prevalence of about 1% among Caucasians (Harris, B. J. *et al.*, 1997, In Textbook of Rheumatology 898-932), currently affecting 2.5 million Americans. RA is characterized by chronic
- 25 inflammation of the synovial joints and infiltration by activated T cells, macrophages and plasma cells, leading to a progressive destruction of the articular cartilage. It is the most severe form of joint disease.
- [00235]** In still another aspect, the autoimmune disease treated with the antagonistic anti-CCR2 antibodies is Multiple Sclerosis (MS). MS, also a TH1
- 30 disorder, is the most common central nervous system (CNS) demyelinating disease, affecting 350,000 (0.1%) individuals in North America and 1.1 million worldwide. In general, MS is considered to be an autoimmune disease mediated in part by proinflammatory CD4 T (Th1) cells and monocytes that recognize

specific myelin polypeptides in association with MHC class II molecules expressed on antigen (Ag) presenting cells (APC).

5 [00236] The antagonistic anti-CCR2 antibodies or antigen-binding fragments thereof (e.g., 4.40 and/or 4.9 or antigen-binding fragments thereof) can be used to treat human type I or insulin-dependent diabetes mellitus (IDDM), a disease characterized by autoimmune destruction of the beta cells in the pancreatic islets of Langerhans. The depletion of beta cells results in an inability to regulate levels of glucose in the blood. In humans a long presymptomatic period precedes the onset of diabetes. During this period there is a gradual loss of pancreatic beta cell
10 function. The development of disease is implicated by the presence of autoantibodies against insulin, glutamic acid decarboxylase, and the tyrosine phosphatase IA2 (IA2).

[00237] The antagonistic anti-CCR2 antibodies or antigen-binding fragments thereof (e.g., 4.40 and/or 4.9 or antigen-binding fragments thereof) also can be
15 used to treat neuropathic pain. Mice lacking the CCR2 have been shown to have reduced neuropathic pain (Abbadie *et al.*, *Proc Natl Acad Sci U S A.* 100(13): 7947-52 (2003)). As used herein, the term "neuropathic pain" means pain resulting from injury to a nerve. Neuropathic pain is distinguished from nociceptive pain, which is the pain caused by acute tissue injury involving small cutaneous
20 nerves or small nerves in muscle or connective tissue. Pain involving a nociceptive mechanism usually is limited in duration to the period of tissue repair and generally is alleviated by available analgesic agents or opioids as described in Myers, *Regional Anesthesia* 20:173-184 (1995). Neuropathic pain typically is long-lasting or chronic and often develops days or months following an initial
25 acute tissue injury. Neuropathic pain can involve persistent, spontaneous pain as well as allodynia, which is a painful response to a stimulus that normally is not painful. Neuropathic pain also can be characterized by hyperalgesia, in which there is an accentuated response to a painful stimulus that usually is trivial, such as a pin prick.

30 [00238] The antagonistic anti-CCR2 antibodies are useful in alleviating neuropathic pain resulting from a disorder of peripheral nerve, dorsal root ganglia, spinal cord, brainstem, thalamus or cortex. The method is useful in alleviating neuropathic pain regardless of the etiology of the pain. For example, a method

can be used to alleviate neuropathic pain resulting from a peripheral nerve disorder such as neuroma; nerve compression; nerve crush, nerve stretch or incomplete nerve transection; mononeuropathy or polyneuropathy. A method also can be used to alleviate neuropathic pain resulting from a disorder such as dorsal root ganglion compression; inflammation of the spinal cord; contusion, tumor or hemisection of the spinal cord; tumors of the brainstem, thalamus or cortex; or trauma to the brainstem, thalamus or cortex

[00239] The antagonistic anti-CCR2 antibodies or antigen-binding fragments thereof (e.g., 4.40 and/or 4.9 or functional fragments thereof) can be also be used to treat atherosclerosis. Atherosclerotic plaque develops over several decades and involves inflammatory cell infiltration, smooth muscle cell proliferation, accumulation of extracellular matrix, fibrous cap formation, and angiogenesis. (Bayes-Genis *et al. Circ. Res.* 86:125-130 (2000)). Chemotaxis is involved in the early development of atherosclerosis. Cell populations migrate toward the inner part of the vascular wall and originate the neointima, which leads to the formation of an atherosclerotic plaque. For example, monocyte chemotaxis is induced by monocyte chemoattractant protein 1 (MCP-1), which is expressed early in the development of atherosclerosis in the injured arterial wall. (Furukawa *et al. Circ. Res.* 84:306-314 (1999); Han *et al. J. Lipid Res.* 40:1053 (1999)). Transplantation of bone marrow from CCR2 ^{-/-} mice, but not from CCR2 ^{+/+} mice, into ApoE3-Leiden mice, a mouse strain susceptible for diet-induced atherosclerosis, decreases atherogenesis, suggesting that MCP-1 signaling through CCR2 contributes to atherosclerosis (Guo *et al.* (2003) *Arterioscler Thromb Vasc Biol.*;23(3):447-53). Accordingly, the CCR2 antagonist antibodies and in particular the antagonist antibodies which bind to the first and/or second extracellular loop of CCR2, may be administered to a subject to reduce the incidence of, to treat, or to aid in the treatment of atherosclerosis.

[00240] The antagonistic anti-CCR2 antibodies or antigen-binding fragments thereof (e.g., 4.40 and/or 4.9 or antigen-binding fragments thereof) also can be used to treat obesity. In obesity, adipose tissue has been demonstrated to contain large numbers of monocytes. These monocytes may contribute either to fat deposition or the development of various sequelae commonly associated with

Obesity commonly termed metabolic syndrome. Metabolic syndrome includes such alterations as the development of diabetes.

5 [00241] The antagonistic anti-CCR2 antibodies or antigen-binding fragments thereof (e.g., 4.40 and/or 4.9 or antigen-binding fragments thereof) can be also be used to treat stenosis or restenosis of the vasculature, particularly of the arteries, e.g., the coronary artery, such as stenosis or restenosis which results from vascular intervention (e.g., surgical, therapeutic or mechanical intervention), as well as neointimal hyperplasia. For example, restenosis, which typically produces a narrowing of the luminal opening of the vessel, can result from vascular injury
10 including, but not limited to, that produced by vascular graft procedures, angioplasty, including angioplasty performed by balloon, atherectomy, laser or other suitable method (e.g., percutaneous transluminal coronary angioplasty (PTCA)), stent placement (e.g., mechanical or biological endovascular stent placement), vascular bypass procedures or combinations thereof, as well as other
15 procedures used to treat stenotic or occluded blood vessels.

[00242] The antagonistic anti-CCR2 antibodies or antigen-binding fragments thereof (e.g., 4.40 and/or 4.9 or antigen-binding fragments thereof) also can be used to treat graft rejection (e.g., in transplantation), including allograft rejection or graft-versus-host disease, and organ transplant-associated arteriosclerosis.

20 [00243] Antibodies and antigen-binding fragments thereof which are antagonists of CCR2 can be used as therapeutics for HIV infection. HIV-1 and HIV-2 are the etiologic agents of acquired immunodeficiency syndrome (AIDS) in humans. AIDS results in part from the depletion of CD4⁺ T lymphocytes in HIV infected individuals. HIV-1 infects primarily T lymphocytes, monocytes/macrophages,
25 dendritic cells and, in the central nervous system, microglia. All of these cells express the CD4 glycoprotein, which serves as a receptor for HIV-1 and HIV-2. Efficient entry of HIV into target cells is dependent upon binding of the viral exterior envelope glycoprotein, gp120, to the amino-terminal CD4 domain.

[00244] Furthermore, CCR2 has been shown to act as a co-receptor for HIV-1
30 (Frade *et al.* (1997) *J Clin Invest.*;100(3):497-502). After virus binding, the HIV-1 envelope glycoproteins mediate the fusion of viral and host cell membranes to complete the entry process. Membrane fusion directed by HIV-1 envelope

glycoproteins expressed on the infected cell surface leads to cell-cell fusion, resulting in syncytia.

[00245] Antibodies and antigen-binding fragments thereof which are antagonists of CCR2 can be used as therapeutics for the treatment of a variety of ophthalmology conditions including Age Related Macular Degeneration (AMD), Uveitis, and Corneal Infections.

Age Related Macular Degeneration (AMD)

[00246] CCR2 has been shown to play role in the recruitment of macrophages in the development of choroidal neovascularization (CNV) that is observed in age related macular degeneration patients, as well as in angioid streaks, high myopia, ocular histoplasmosis patients (Tsutsumi, C. et al. *Journal of Leukocyte Biology* 74:25-32, 2003).

Uveitis

[00247] An association between single nucleotide polymorphisms of CCR2 and its ligand (MCP-1) has been demonstrated in patients with acute idiopathic anterior uveitis (Yeo, TK, et al. *Cytokine* 35:29-35 2006) and idiopathic immune-mediated posterior segment uveitis (Ahad, MA, et al. *Mol Vis* 13:388-396 2007)

Corneal Infections (bacterial):

[00248] CCL2 has been shown to play a role in the regulation of polymorphonuclear neutrophils (PMNs) recruitment during corneal infection (Xue, M. L, et al. *Immunology and Cell Biology* 85:525-531 2007). While PMNs are essential for eliminating bacteria and promoting wound healing in the cornea the persistence of these cells may result in chronic inflammatory disease.

[00249] Antibodies and antigen-binding fragments thereof which are antagonists of CCR2 can be used as therapeutics for the treatment of a variety of solid tumors and cancers, including chronic lymphocytic leukemia, chronic myelocytic leukemia, multiple myeloma, malignant myeloma, Hodgkin's disease, and carcinomas of the prostate, bladder, breast, cervix, colon, lung, liver or stomach solid tumors and cancers.

Prostate Cancer

[00250] MCP-1 has been shown to act as a paracrine and autocrine factor for prostate cancer growth and invasion (Lu, Y. et al. *The Prostate* 66:1311-1318, 2006) and CCR2 expression has been shown to correlate with prostate cancer progression (Lu, Y. et al. *Journal of cellular biochemistry* 101:676-685, 2007). Systemic delivery of neutralizing anti-MCP-1 antibodies has been shown to induce prostate cancer tumor regression in mice (Loberg, R. D., *Cancer Res* 67:9417-9424 2007).

Breast Cancer

[00251] Tumor associated macrophages are thought to play a critical role in tumor immune surveillance and development and the activation and recruitment of lymphocytes are regulated by chemokines including MCP-1. A significant association has been demonstrated for CCR2 polymorphism in breast cancer (Zafiroopoulos, A., N. et al. *Journal of medical genetics* 41:e59 2009). The CCL2/CCR2 pathway has also been shown to play a pivotal role in the recruitment to cancers, including breast, ovarian and gastric cancers, of myeloid suppressor cells, which promote tumor progression, angiogenesis and vasculoangiogenesis (Huang, B., et al. *Cancer letters* 252:86-92, 2007).

Melanoma

[00252] Blocking of MCP-1 function has also been shown to inhibit the recruitment of tumor associated macrophages and prevent tumor angiogenesis and growth in malignant melanoma in mice (Koga, M. et al. *Biochemical and biophysical research communications* 365:279-284, 2008).

Liver Cancer

[00253] Blocking of CCR2 has been shown to reduce trafficking of hepatic stellate cells, a main source of matrix metalloproteinase 2, which facilitates neovascularization during liver tumor formation (Yang, X. et al. *International journal of cancer* 118:335-345, 2006).

Cervical Cancer

[00254] CCR2 has been shown to play a role in macrophage recruitment leading to tumor angiogenesis in the development of cervical neoplasia from squamous intraepithelial lesions (Coelho, A., et al. *Gynecologic oncology* 96:760-764, 2005; 5 .Coelho, A., et al. *Gynecologic and obstetric investigation* 64:208-212, 2007).

Ovarian Cancer

[00255] The CCL2/CCR2 pathway has also been shown to play a pivotal role in the recruitment to cancers, including breast, ovarian and gastric cancers, of 10 myeloid suppressor cells, which promote tumor progression, angiogenesis and vasculoangiogenesis (Huang, B., et al. *Cancer letters* 252:86-92, 2007).

[00256] The antibody may be administered once or multiple times. The antibody may be administered from four times daily to once every six months or longer. 15 The administering may be on a schedule such as three times daily, twice daily, once daily, once every two days, once every three days, once weekly, once every two weeks, once every month, once every two months, once every three months and once every six months. The antibody may also be administered continuously via a minipump. The antibody may be administered via a mucosal, buccal, 20 intranasal, inhalable, intravenous, subcutaneous, intramuscular, parenteral, intratumor or topical route. The antibody may be administered locally or systemically.

[00257] The antibody may be administered once, at least twice or for at least the period of time until the condition is treated, palliated or cured. The antibody will 25 generally be administered as part of a pharmaceutical composition as described herein. The dosage of antibody will generally be in the range of 0.1-100 mg/kg, 0.5-50 mg/kg, 1-20 mg/kg, or 1-10 mg/kg. The serum concentration of the antibody may be measured by any method known in the art.

[00258] The examples below are for illustration only and are not to be construed 30 as limiting the scope in any manner.

EXAMPLE 1

Generation of Hybridomas Producing Anti-CCR2 Antibody

[00259] Eight to ten week old XENOMOUSE™ mice that produce human IgG2 and IgG4 antibodies were immunized in their hind footpads with 300-19 cells
5 transfected with CCR2B (Genbank MN000648, SEQ ID NO:204) (10^7 cells/dose/mouse in TITERMAX™ Gold Adjuvant, Sigma, Catalog # T2684, lot # K1599; prepare 50/50 volume). The mice received five to nine booster injections in Aluminium Phosphate Gel Adjuvant (Catalog # 1452-250, batch #8937, HCl Biosector (5ul/mouse/boost)) and qCpG (IMMUNEASY™ Mouse Adjuvant)
10 (Catalog # 303101; lot #11551249; Qiagen (15ul/mouse/boost)) over a three to eight week period. Four days before fusion, the mice were given a final injection in PBS. The spleen and lymph node lymphocytes from immunized mice were collected and fused with the non-secretory myeloma P3-X63-Ag8.653 cell line. The fused cells were subjected to HAT selection as previously described (Galfre
15 and Milstein, *Methods Enzymol.* 73:3-46 (1981)). A panel of hybridomas all secreting CCR2-specific human antibodies was recovered. A number of antibodies were identified for binding to CCR2 as assessed by FACS analysis. Hybridomas were selected for further study, some of which are listed in Table 6.
[00260] The hybridomas indicated in Table 2 were deposited under terms in
20 accordance with the Budapest Treaty with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, VA 20110-2209. The hybridomas have been assigned the following accession numbers:

Table 2

Antibody	Mouse Hybridoma Cell Line Designation	Strain Designation	ATCC Designation	Deposit Date
4.9.3	PF11-4.9.3	LN 15923	PTA-6979	Sept. 16, 2005
4.22.3	PF11-4.22.3	LN 15924	PTA-6980	Sept. 16, 2005
4.40.3	PF11-4.40.3	LN 15925	PTA-6981	Sept. 16, 2005
7.123.1	PF11-7.123.1	LN 15931	PTA-7341	Jan. 31, 2006
8.19.1.1	PF11-8.19.1.1	LN 15932	PTA-7342	Jan. 31, 2006

EXAMPLE 2Sequencing of Anti-CCR2-Antibodies

- 5 [00261] To analyze the structure of antibodies produced, nucleic acids were cloned that encode heavy and light chain variable domain containing fragments from hybridomas producing anti-CCR2 monoclonal antibodies. The light chains and the light chains of the CCR2 antibodies were cloned and sequence verified as exemplified for the 4.40.2 antibody as follows:
- 10 Poly(A)⁺ mRNA was isolated using an RNEASYTM MINI KIT (Qiagen) and cDNA synthesized from the mRNA with the ADVANTAGE RT-for-PCR kit (BD Biosciences) using oligo(dT) priming. The oligo(dT) primed cDNA was amplified for clone 4.40.2 using the primers listed in Table 3. Amplification was achieved using the PFUULTRA HIGH-FIDELITY Polymerase (Stratagene) and a PTC-200
- 15 DNA Engine (MJ Research) with cycling as follows: 2' at 95°C; 25X (20" at 95°C, 30" at 55°C, 30" at 72°C); 10' at 72°C. PCR amplicons were cloned into the heavy and light chain expression vectors. Vectors were then transformed into MAX EFFICIENCY DH5 α chemically competent cells (Invitrogen) using the standard protocol. Clones were sequence verified using Grills 16th BDTv3.1/dGTP
- 20 chemistry (Applied Biosystems Inc) and a 3730xl DNA Analyzer (Applied Biosystems Inc). From the nucleotide sequence and predicted amino acid sequence of the antibodies, the gene usage was identified for each antibody

chain. Alignments to the "V Base sequence directory" (MRC Centre for Protein Engineering, Cambridge, UK; Tomlinson, et al., J. Mol. Biol., 227, 776–798 (1992); Hum. Mol. Genet., 3, 853–860 (1994); EMBO J., 14, 4628–4638 (1995)) were made using MACVECTOR™ and GENEWORKS™ software programs (Oxford Molecular Group, Campbell, CA, USA).

Table 3: Variable Domain Primers Designed for Amplifying 4.40.2 (5' to 3')

CCR2_440_VH_G2_F	VH3.30	CAGGTGCAGCTGGTGGAGTCTGG (SEQ ID NO:147)
CCR2_440_VH_G2_R	JH3b	GAAGAGACGGTGACCATTGTCCCTT (SEQ ID NO:148)
CCR2_440_VL_K_F	A26	GAAATTGTGCTGACTCAGTCTCCAGAC (SEQ ID NO:149)
CCR2_440_VL_K_R	JK4	GTTTGATCTCCACCTTGGTCCCTC (SEQ ID NO:150)

[00262] Table 4 sets forth the gene utilization of selected hybridoma antibody clones.

Table 4

Heavy and Light Chain Gene Utilization

Clone	Heavy Chain Germline			Kappa Light Chain Germline	
	V _H	D _H	J _H	V _K	J _K
4.9.3	VH3-30	D1-7	JH3B	O12	JK2
4.22.3	VH1-46	D1-7	JH3B	A1	JK5
4.39.3	VH1-46	D1-7	JH3B	B3	JK1
4.40.3	VH3-30	D1-7	JH3B	A26	JK4

[00263] Hybridoma clones were generated in IgG4 mice and once the sequences were obtained the variable domains were cloned into IgG1, IgG2, and IgG4 format expression vectors for comparison.

EXAMPLE 3

Mutagenesis of Anti-CCR2-Antibodies

Reversion to germline sequence in light chain variable region

5 [00264] The alanine residue at position 68 in the light chain of CCR2 4.40.2 was reverted to the germline residue, glycine, by site-directed mutagenesis as follows. The point mutation in the codon for amino acid residue 68 was changed using the primers in Table 5. Site-directed mutagenesis was performed using a QUIKCHANGE™ II Site-Directed Mutagenesis kit (Stratagene) with the standard protocol including the following modifications to cycling: 2' at 96°C; 16X (50" at 96°C, 10" at 68°C); 10' at 68°C. Mutagenized vector was transformed into XL1-Blue supercompetent cells. To verify that mutagenesis was successful, clones were sequenced using Grills 16th BDTv3.1/dGTP chemistry (Applied Biosystems Inc.) and a 3730xl DNA Analyzer (Applied Biosystems Inc.).

Table 5: Mutagenesis Primers (5' to 3')

CCR2_440_VK_G68A_F	GGCAGTGGATCTGGGACAGATTTCACC (SEQ ID NO:151)
CCR2_440_VK_G68A_R	GGTGAAATCTGTCCAGATCCACTGCC (SEQ ID NO:152)

15 *Changed nucleotide is bolded and underlined.

[00265] The resulting antibody was designated 4.40 A68G (SEQ ID NO:112), having a glycine at position 68 of the light chain variable region.

20 Generation of IgG4 heavy chain constant region with hinge-stabilizing mutation

[00266] The IgG4 heavy chain constant regions were isolated from a pCON-G4(pro) (Lonza, Basel, Switzerland) derived IgG4 expression vector, which has a hinge region stabilizing mutation from germline, Serine to Proline at amino acid 230 (Angal, S. et al. *Molecular Immunology* 30:105-108 (1993)), in the context of the IgG4 L309 allotype (Brusco A. et al., *Eur J. Immunogenetics* 25:349-355 (1998)).

[00267] A silent mutation (Lys, AAG→AAA) was introduced by PCR mutagenesis into the 11th nucleotide residue of the G4 CH1 exon of pCON-G4(pro) vector, to

remove an Apal recognition site to facilitate subcloning, using the following amplification primers:

G4_Forward

ttatgctgggcccagctctgtcccacaccgcggtcacatggcaccacctctcttgcaGCTTCCACC

5 AAAGGCCCATCCGTCTTCCCC (SEQ ID NO:153)

(Hybridizing bases in CAPS (mutation of residue #11 of CH1 is BOLDED); Non-hybridizing bases in lowercase)

G4_Reverse:

tcatattctctagaTCATTTACCCAGAGACAGGGAGAGG (SEQ ID NO:154)

10 (Hybridizing bases in CAPs. Non-hybridizing bases with XbaI site in lowercase)

[00268] Amplification was achieved using Expand Hi-Fi Polymerase (Roche) and a PTC-200 DNA ENGINE™ (MJ Research) with thermal cycling as follows: 3' at 95°C; 22X (20" at 95°C, 30" at 58°C, 2'10" at 72°C); 10' at 72°C.

[00269] Another silent mutation (Thr, ACC→ACA) was introduced by PCR
15 mutagenesis at nucleotide residue 209 of the G4 CH1 exon of the pCON-G4(pro) vector to remove a BstEII site in order to facilitate use of that enzyme for subcloning variable regions into the expression vector using the following primers:

G4delBst_F

CAGCGTGGTGACAGTGCCCTCCAGCAG (SEQ ID NO:155)

20 G4delBst_R

CTGCTGGAGGGCACTGTCACCACGCTG (SEQ ID NO:156).

[00270] Amplification was achieved using the QUIKCHANGE™ Site-Directed Mutagenesis Kit (Stratagene) and a PTC-200 DNA Engine (MJ Research) with thermal cycling as follows: 1' at 96°C; 14X (50" at 96°C, 15'48" at 68°C); 10' at
25 72°C.

[00271] The resulting IgG4 constant region comprising the hinge region mutation and the two silent mutations, referred to as S230P, was then subcloned as an Apal/XbaI fragment into the Apal/XbaI sites of a DHFR antibody expression vector. The heavy chain variable region of the 4.40.2 antibody was then cloned
30 into the expression vector comprising the G4 S230P constant region resulting in a full-length heavy chain construct. The 4.40 derived antibody having the light chain variable region A68G germline substitution and the heavy chain hinge region S230P substitution was designated 4.40 A68G S230P having the light chain

amino sequence of SEQ ID NO:112 and the heavy chain amino acid sequence of SEQ ID NO:116.

5 [00272] Supernatants from cells transfected with the expression vectors were collected and purified by standard Protein A affinity chromatography to isolate recombinant immunoglobulins. These proteins were then characterized by SDS-PAGE, light scatter, and spectrophotometry.

EXAMPLE 4

In vitro binding

10 [00273] Figure 1 shows the *in vitro* binding of AF-488 conjugated antibody 4.40 A68G S230P to human monocytes in whole blood (Figure 1A), to CCR2-transfected 300-19 cells (Figure 1B), and binding of different concentrations of 4.40 A68G S230P antibody to CCR2-transfected 300-19 cells as detected with anti-human PE (Figure 1C). Briefly, 1 million cells in a volume of 100ul were
15 stained with the test antibody using Dulbecco's Phosphate Buffered Saline, containing 2% heat inactivated Fetal Bovine Serum and 0.002% Sodium Azide. Fifteen minutes later, a secondary detection antibody was added. After 30 minutes, cells were washed in buffer, resuspended in 500ul and assessed for staining on a FACS CALIBUR. A total of 10,000 events per tube were assessed.
20 The mean channel fluorescent intensity was assessed for each concentration of antibody as an indicator for the magnitude of staining.

EXAMPLE 5

Determination of Affinity Constants (K_D) of Anti-CCR2 Monoclonal Antibodies

25 [00274] To assess the K_D of the antibodies for the CCR2, antibody binding to 300-19 cells expressing CCR2 was assessed by FACS in a 3 hour assay. Briefly, using a buffer of Dulbecco's Phosphate Buffered Saline containing 2% heat inactivated Fetal Bovine Serum, 0.002% Sodium Azide and 0.005mg/ml Cytochalasin-B (Sigma 6762), 1 million cells in a volume of 100ul were stained
30 with the test antibody. Fifteen minutes later, a secondary detection antibody, R-Phycoerythrin conjugated Affini-pure F(ab') fragment Donkey anti-human IgG H+L (Jackson 709-116-149), was added. Tubes were gently shaken for 3 hours at room temperature. After 2 washes in buffer, cells were resuspended in 500ul of

buffer and assessed on a FACS CALIBUR™. A total of 10,000 events per tube were assessed. The mean channel fluorescent intensity was assessed for each concentration of antibody as an indicator for the magnitude of staining. These binding studies demonstrated that the 4.40 A68G S230P antibody binds to human CCR2 on transfected cells with a K_D of 0.085 ug/mL (0.58nM) (Figure 2). When saturation binding was assessed on cells expressing a CCR2/CCR5 chimera receptor having the first and second extracellular loops of the CCR2 (see Example 7), a similar concentration curve and K_D (K_D = 0.023 ug/mL (0.16nM)) were obtained as shown in Figure 6C.

EXAMPLE 6

Inhibition of MCP-1 induced Chemotaxis

[00275] Human anti-CCR2 antibodies were evaluated for their ability to inhibit the chemotaxis of THP-1 monocytes (ATCC # TIB 202), in response to the CCR2 ligand, MCP-1 (CCL2). Chemotaxis was conducted in 96 well chemotaxis chambers purchased from NeuroProbe, Inc. (Gaithersburg, MD) as previously described (see Gladue RP *et al.*, *J Biol Chem* 278:40473-40480 (2003)). Briefly, CCL2 was diluted in RPMI 1640 medium (Roswell Park Memorial Institute) containing 0.1% BSA, and then added to the bottom wells of the chamber. A filter with 5 μ m pores (Neuroprobe) was placed between the upper and lower wells of the chamber. THP-1 cells were then added to the top chamber (8×10^5) in the presence or absence of various concentrations of the test antibodies. The apparatus was incubated for 3 hrs in a 5% CO₂ humidified incubator at 37°C. After the incubation period, the non-migrating cells were removed from the upper chamber and the top of the filter was wiped. 2 mM cold EDTA was then added to the upper wells, and the chemotaxis chamber was incubated at 4°C for 20 minutes. The EDTA was then removed and the 96 well microtiter plate was centrifuged at 800 x g for 10 minutes. The filter was then removed, the culture medium discarded, and 0.2% FDA was added to the plates. The plates were then incubated for 1.5 hours at 37°C until a yellow color developed. The number of migrating cells was quantified by reading the intensity of the color on a microtiter plate reader at 490 nm. As shown in Table 6, several antibodies that inhibited THP1 chemotaxis to different degrees were identified.

Table 6: Inhibition of MCP-1-induced THP-1 Chemotaxis

Antibody Clone	IC50 (ug/ml)
4.40.3	0.137
4.22.3	0.138
4.39.3	0.139
4.9.2	0.198
4.6.3	0.400
4.48.3	0.491
4.41.1	0.521
4.3.1	0.618
4.52.1	0.641
4.59.2	1.020
4.24.3	2.264

- 5 [00276] Similarly, the antibody 4.40 A68G S230P inhibited chemotaxis of THP-1 monocytes in response to MCP-1 (IC50 = 0.148 ug/ml) but not the CCR1/CCR5 ligand MIP-1, as shown in Figure 3.

Primary Monocyte Chemotaxis

- 10 [00277] Heparinized human whole blood was layered over ACCUSPIN HISTOPAQUE 1077 tubes (Sigma; St. Louis, MO) and spun down. The mononuclear cell fraction was collected and washed 3X with PBS, red blood cells (RBC's) were lysed with water, and the cells were resuspended at 4×10^6 /ml in RPMI (Gibco; Grand Isle, NY) with 0.1% BSA (Sigma) and 10 mM HEPES
- 15 (Gibco). Antibody dilutions or KLH control were added to 0.25 nM MCP-1 (Peprotech; Rocky Hill, NJ) and 30 ul were placed in the bottom of a 48 well Boyden chamber (Neuroprobe; Gaithersburg, MD); negative control was media alone. A 5 um PVP-free filter (Neuroprobe) was placed over this and the chamber sealed. Isolated mononuclear cells were incubated with antibody dilutions or KLH
- 20 control for 30 minutes at room temperature. Then 50 ul were added to the upper wells. The chamber was incubated for 90 minutes at 37°C in a 5% CO₂ humidified incubator. After incubation, the cells were aspirated out of the upper wells, the top of the filter was wiped, air-dried, stained with DIFF-QUIK™ (Dade-Behring; Newark, DE), and the number of migrating cells in 6 fields were counted.

The inhibition of chemotaxis of primary isolated human monocytes in response to MCP-1 by antibody 4.40 A68G S230P is shown in Figure 4.

[00278] Further, as exemplified with the 4.40 antibody, no inhibition of THP-1 cells was observed to the CCR1/CCR5 ligand MIP-1a (Figure 3).

5

EXAMPLE 7

CCR2/CCR1 Chimera Construction

[00279] Antibodies that inhibited MCP-1-induced chemotaxis were tested for binding to CCR2 chimeras to map their epitopes. This was accomplished by
10 assessing the binding of the antibodies to 300-19 cells expressing different receptor chimeras consisting of extracellular loop (1st, 2nd and/or 3rd) and N-terminal substitutions of the CCR2 with portions of the CCR1 (M = CCR2, R = CCR1).

15 Construction of chimera receptor MRRR

[00280] The MRRR chimeric receptor (N-terminus of CCR2 (M), 1st, 2nd, and 3rd extracellular loop of CCR1 (R) was constructed using the QUIKCHANGE® Site-Directed mutagenesis kit from Stratagene and the following mutagenesis primers to mutate the first ApaI restriction enzyme site in wild type human CCR1, cloned
20 into the expression vector pcDNA3 (Invitrogen, Carlsbad, CA) to a BamHI site using the following primers:

sense primer 40A:

CGAGAGGGCCTTTGGGATCCAACTGCTGCC (SEQ ID NO:132)

anti-sense primer 40B:

25 GGCAGCAGTTGGATCCCAAAGGCCCTCTCG (SEQ ID NO:133)

[00281] The chimeric receptor containing a FLAG-tagged MMRR chimera (N-terminus and 1st extracellular loop of CCR2, 2nd and 3rd extracellular loops of CCR1) cloned into pCMV-1 derived expression vector (Pharmacia; Piscataway,
30 NJ) was provided by Israel Charo of the J. David Gladstone Institutes. The construction of the MMRR chimera (referred to as "2211" by the authors) is described in Montecarlo F. S. et al. (*Methods in Enzymology* 228:70-84 (1997)).

The first ApaL site in the chimera was mutated to a BamHI site using the following mutagenesis primers:

Sense primer: 41A:

GCAAATTGGGATCCAACTCCTGCC (SEQ ID NO:134)

5

Anti-sense primer: 41B:

GGCAGGAGTTGGATCCCAATTTGC (SEQ ID NO:135).

[00282] Each resulting mutated clone was cut with NdeI and BamHI. The fragment containing the vector from the CCR1/pcDNA3 BamHI mutant and the 632 basepair NdeI/BamHI fragment containing just the CCR2 N-terminus from the mutated MMRR/pCMV1 plasmid were gel isolated and ligated together resulting in the MMRR/pcDNA3 chimera.

[00283] The BamHI site in the MMRR chimera was mutated back to an ApaL site using the following mutagenesis primers:

Sense primer 41C:

15

GCAAATTGGGGCCCAACTGCTGCC (SEQ ID NO:136)

Anti-sense primer 41D:

GGCAGCAGTTGGGCCCCAATTTGC (SEQ ID NO:137).

[00284] The complete MMRR fragment was PCR amplified using sense primer 66C (SEQ ID NO:138) homologous to the FLAG tag region of the vector and anti-sense primer 66D (SEQ ID NO:139) homologous to the 3' end of CCR1 and containing a HindIII site.

Sense primer 66C

CTCTTGTGCCAGGGTGTGGTCTCCGA (SEQ ID NO:138)

Anti-sense primer 66D

25

GATCGAAGCTTTTCAGAACCCAGCAGAGAGTTCATG (SEQ ID NO:139)

[00285] This fragment was then subcloned into the PflMI and HindIII sites of a pMIG (Van Parijs, L. et al., *Immunity* 11:281-188 (1999)) derived retroviral construct (Figure 5), replacing an inserted gene (a mouse/human chimera) that was preceded by a FLAG tag followed by the SalI site and had the internal ribosomal entry site—enhanced green fluorescent protein site (IRESEGFP) removed.

Construction of chimera receptor RRRM and RMMR

5 [00286] The RRRM (N-terminus, 1st and 2nd extracellular loops of CCR1, and 3rd extracellular loop CCR2) and RMMR (N-terminus and 3rd extracellular loop CCR1, and 2nd and 3rd extracellular loops of CCR2) chimeric receptors were made using a two step PCR method.

[00287] For the RRRM chimera, the third loop of human wild type CCR2 was first PCR amplified using the following primers:

Sense chimeric primer 89A:

GACTATACTTATTTCTGTTTTCATTGTCATTCTCCTGAACACC (SEQ ID NO:140)

10 Anti-sense primer 81B:

CGCCAAGCTTCATTATAAACCAGCCGAGA (SEQ ID NO:141).

The wild type human CCR2 was cloned into retroviral-derived expression vector pMIG as a template. The N-terminal region through the second extracellular loop was PCR amplified using the following primers:

15 Sense primer 89C:

ACGCGTCGACGAAACTCCAAACACCACAGAG (SEQ ID NO:142)

Anti-sense chimeric primer 89B:

GTTTCAGGAGAATGACAATGAAAACAGAAATAAGTATAGTC (SEQ ID NO:143),

and wild type CCR1 was cloned into the expression vector pcDNA3 as a template.

20 [00288] These fragments were gel purified, combined and PCR-amplified together using the 5' and 3' end primers: 89C (SEQ ID NO:142) and 81B (SEQ ID NO:141). The resulting fragment was ligated into the Sall/HindIII sites of the retroviral vector, pMIG containing an N-terminal FLAG tag and having the IRESEGFP removed.

25

Construction of the chimera receptor RMMR

[00289] The CCR1 third extracellular loop through the cytoplasmic tail was PCR-amplified using wild type CCR1 cloned into the expression vector pcDNA3 as a template and the following primers:

30 Sense chimeric primer 85A:

TCATTCTCCTGAACACCTTCCAAGACTTCCTGTTACCCCA (SEQ ID NO:144)

Anti-sense primer 78D:

GCCAAGCTTCCAGTGTGATGGATATCTGA (SEQ ID NO:145),

which hybridizes to an area of the vector 3' of the insert in the wild type CCR1/pcDNA3 plasmid.

5 [00290] A second fragment was PCR-amplified containing the N-terminus of CCR1 and an area containing the first and second extracellular loops of CCR2 using a FLAG tagged RMMM/pcDNA3 plasmid obtained from Israel Charo of the J. David Gladstone Institutes as a template. The construction of the RMMM chimera construct (referred to as "2111" by the authors) is described in Montecarlo, F. S. et al. (*Methods in Enzymology* 228:70-84 (1997)). The primers used were sense primer 66C (SEQ ID NO:138), which hybridizes to the FLAG tag
10 and the anti-sense chimeric primer 85B:

TGGGTGAACAGGAAGTCTTGGAAGGTGTTTCAGGAGAATGA (SEQ ID NO:146) .

These two fragments were gel purified, combined and PCR-amplified together using the 5' and 3' end primer: 66C (SEQ ID NO:138) and 78D (SEQ ID NO:144). This final fragment was digested with PflMI and HindIII and ligated into the pMIG
15 retroviral vector containing a N-terminal FLAG tag and having the IRESEGFP removed.

Chimera expression

[00291] Retrovirus was produced from each chimera construct and used to
20 transduce 300-19 cells. Expression was determined by FACS analysis using anti-FLAG epitope antibody M1 (Sigma; St. Louis, MO, catalog # F3040).

EXAMPLE 8

Epitope mapping

25 [00292] Binding of antibodies to the CCR2/CCR1 chimeric receptors was assessed using FACS analysis. All receptors were tagged at the N-terminus with FLAG and also stained with the anti-FLAG antibody M1 such that receptor expression could be confirmed. Figures 6A & B illustrate the FACS staining of FLAG on the N-terminus with M1 antibody (Figure 6A) as compared to the
30 staining of 4.40 (Figure 6B). Figure 6C illustrates a saturation binding curve of the 4.40 A68G S230P antibody on RMMR chimera transfected 300-19 cells. Saturation binding of the 4.40 A68G S230P antibody to 300-19 cells not transfected with CCR2 showed no binding at concentrations up to 10 ug/mL

(Figure 7A) while 300-19 cells transfected with fully human CCR2 displayed dose-dependent binding at concentrations above 0.01 ug/mL (Figure 7B). Moreover, saturation binding of the 4.40 A68G S230P antibody to chimeric receptors expressing only the N-terminus of CCR2 (MRRR) together with the 3 loop regions of CCR1 (Figure 7C) or the 3rd extracellular loop of CCR2 with the N-terminus and 1st and 2nd loop regions of CCR1 (RRRM) failed to exhibit any significant binding at concentrations up to 10 ug/mL (Figure 7D). By contrast, saturation binding of the 4.40 A68G S230P antibody to chimeric receptors expressing the N-terminus and 3rd loop region of CCR1 and the 1st and 2nd loop regions of CCR2 (RMMR) showed significant dose-dependent binding at concentrations above 0.001 ug/mL (Figure 7E). Receptor expression on all of these receptor transfectants was confirmed by assessing the staining of FLAG on the N-terminus with an M1 antibody (Sigma #F3040).

Table 7: Epitope mapping

Clone	Full Length CCR2 (MMMM)	N-terminus of CCR2 (MRRR)	1 st , 2 nd , and 3 rd loops of CCR2 (RMMM)	1 st and 2 nd loops of CCR2 (RMMR)	Chemotaxis IC50 (µg/ml)	Epitope
4.40.2	+	-	+	+	0.130	1 st /2 nd loop
4.9.2	+	-	+	+	0.198	1 st /2 nd loop
4.52.3	+	+	-		0.642	N-terminus
4.22.3	+	-	+	-	0.137	3 rd loop
4.6.3	+	+	+		0.400	complex*
4.48.3	+	+	+		0.491	complex
4.3.1	+	+	+		0.618	complex
4.59.2	+	+	+		1.020	complex
4.41.1	+	-	-		0.521	complex
4.24.3	+	-	-		2.264	complex
4.39.3	+	-	-	-	0.139	complex

*Complex = binds to wild type but does not bind to the chimeras

Peptide ELISA

[00293] In addition, epitope binding to the first and/or second loop of CCR2 was also assessed using a peptide ELISA. A Reacti-Bind NEUTRAVIDIN™ Coated, High Binding Capacity ELISA plate (Pierce; Rockford, IL) was washed 3X with
5 PBS/0.05% Tween20 and then coated with 100ul/well of 6 ug/ml biotinylated CCR2 loop 2 (SEQ ID NO:129) or loop 3 (SEQ ID NO:130) peptide (AnaSpec; San Jose, CA). The plate was incubated for 1 hour and then washed 3X. The CCR2 antagonist antibody 4.40.3 A68G S230P or other primary antibody, diluted in PBS/0.1% BSA/0.05% Tween20, was then added to the plate in serial dilution
10 and incubated for 1 hour, as were other controls. The plate was washed 3X and 100ul/well HRP-Mouse anti-Human IgG4 secondary antibody (Zymed; So. San Francisco, CA) was added to all the wells at 1:5000 dilution and incubated for 1 hour. The plate was washed 3X and TMB substrate was added to all the wells and incubated for ~30 minutes. The color reaction was stopped with 2M H₂SO₄
15 and the absorbance reading was measured on a plate reader at 450nm (Figure 8). Antibodies, including antibodies 4.9 and 4.40, were identified that bound to the second extracellular loop peptide of the receptor and did not bind to the third loop.

EXAMPLE 9

Binding Selectivity

[00294] The selectivity of the 4.40 A68G S230P antibody for human CCR2 was confirmed by the absence of binding to a closely related chemokine receptor, CCR5, as shown in Figure 9 and by the lack of inhibition of chemotaxis induced by the CCR5/CCR1 ligand MIP-1a (Figure 3). An anti-CCR5 antibody (Pharmingen,
25 catalog #555992) bound to cells expressing CCR5 (Figure 9b) while the CCR2 antibody 4.40.3 A68G S230P showed no binding to these same cells (Figure 9a). For these studies, 300.19 cells engineered to express CCR5 were incubated with either the CCR2 selective antibody, 4.40 A68G S230P, or the CCR5-selective antibody for 30 minutes. Cells were then washed in FACS buffer (0.02% sodium
30 azide, 2% heat inactivated fetal bovine serum in PBS) and analyzed for cell surface expression using a flow cytometer using standard methods.

EXAMPLE 10

Calcium Mobilization

[00295] To determine if antibody 4.40 acts as a functional antagonist on CCR2 and does not possess any significant agonist properties, the effects of 4.40.3 on calcium mobilization of CCR2 transfected 300-19 cells was tested as previously described (Gladue RP *et al.*, *J Biol Chem* 278:40473-40480 (2003)). Human CCR2 transfected 300-19 cells were spun down and resuspended at 2×10^6 cells/ml with PTI buffer (HBSS (Gibco, Grand Island, NY) with 10 mM Hepes (Gibco) and 4.0 mM CaCl_2 (Sigma; St. Louis, MO)). The cells were loaded with 2 ul of indo-1 AM (Molecular Probes; Eugene, OR) per ml (2uM final) and incubated for 25 minutes at 37°C. The cells were then washed 2X with PTI buffer and suspended at 1×10^7 /ml. Several concentrations of the antibody or appropriate controls were added to the cells and incubated for 30 minutes at room temperature. To a 1 mm square cuvette (Sarstedt; Germany), 1.8 ml of pre-warmed PTI buffer were added along with 200 ul of the cell suspension. The cells were excited and fluorescence was measured using equipment from Photon Technology Corporation International (PTI; Lawrenceville, NJ). The machine was paused and 20 ul of 100 nM MCP-1 (Peprotech; Rocky Hill, NJ) was added. After the response, the following reagents were added in this order to release and chelate total calcium: 20 ul of 18% Triton X-100; 20 ul of 3M Tris pH 8.5; 20 ul of 0.5M EGTA pH 8.5 (all from Sigma). The antibody 4.40 inhibited the ability of MCP-1 to induce calcium mobilization in a dose-responsive manner (Figure 10). Similar results were obtained with the 4.40 A68G S230P antibody.

EXAMPLE 11

Inhibition of Chemotaxis Towards CCL2 and CCL7

[00296] To determine if the inhibition of chemotaxis by the 4.40 A68G S230P antibody is specific for MCP-1 or if it also applies to other CCR2 ligands, chemotaxis of THP-1 cells towards MCP-1(CCL2) was compared to that towards CCL3 (MIP-1a) as was shown in Figure 3. In addition the migration of CCR2 transfected 300-19 cells toward another CCR2 ligand, CCL7 (MCP-3) in the presence of antibody 4.40 A68G S230P was also assessed using a similar chemotaxis assay as described in Example 6. While the 4.40 A68G S230P

antibody inhibits chemotaxis towards both known CCR2 ligands, CCL2 (Figure 3) and CCL7 (Figure 11), Figure 3 shows it does not block chemotaxis towards the CCR1/CCR5 ligand, CCL3 (MIP-1a).

5

EXAMPLE 12

Actin Polymerization in Monocytes

Human Whole Blood Actin Polymerization

[00297] Monoclonal Antibody 4.40 A68G S230P also was tested for its ability to inhibit actin polymerization in human whole blood as shown in Figure 12. Blood
10 was collected in EDTA vacutainer tubes (VWR; Boston, MA) and then incubated with dilutions of antibody or KLH control for 30 minutes at room temperature. Buffer control or 10 μ l of 100 nM MCP-1 (Peprotech; Rocky Hill, NJ) were added to a 48 well Corning plate (VWR). 100 μ l of blood was then added with gentle mixing and incubated for 40 seconds after which the reaction was stopped with
15 0.8 ml of stop/lyse reagent (10% of FACS lysing solution (Becton Dickinson; San Jose, CA), 20% of 16% paraformaldehyde (Electron Microscopy Sciences; Ft. Washington, PA), and 70% of H₂O). The plate was incubated for 10 minutes at room temperature, spun down, and cells were transferred to a 96 well round bottom polypropylene plate, and washed 2X with PBS. 100 μ l of PBS were added
20 per well. Then 50 μ l of staining reagent were added (10% of a 5mg/ml lysophosphatidylcholine (Sigma) in 10X HBSS, 80% of the 16% paraformaldehyde, and 10% of 6.6 μ M NBD phalloidin (Molecular Probes; Eugene, OR)). The cells were stained for one hour at room temperature in the dark. After incubation, cells were washed 2X with PBS containing 2% fetal bovine
25 serum (Hyclone, Logan UT) and the fluorescence was quantified on a FACSCAN (Becton Dickinson) gated on monocytes. As shown in Figure 12, antibody 4.40 A68G S230P inhibited actin polymerization with an IC₅₀ of 0.168ug/ml.

Cynomolgus Monkey Whole Blood Actin Polymerization

30 [00298] Blood was collected in EDTA vacutainer tubes (VWR; Boston, MA) and then incubated with dilutions of antibody or isotype control for 30 minutes at room temperature. During this time 10 μ l of 100 nM MCP-1 (Peprotech; Rocky Hill, NJ; final concentration 10 nM) or buffer control (HBSS with no Ca⁺² or Mg⁺² (Gibco;

Grand Island, NY) and 0.2% BSA (Sigma; St. Louis, MO)) were added to a 48 well Corning plate (VWR). 100 ul of blood was then added with gentle mixing and incubated for 40 seconds. The reaction was stopped with 0.8 ml of stop/lyse reagent (10% of FACS lysing solution (Becton Dickinson; San Jose, CA), 20% of
5 16% paraformaldehyde (Electron Microscopy Sciences; Ft. Washington, PA), and 70% of H₂O). The plate was incubated for 10 minutes, spun down, and washed 2X with PBS. The cells were then stained for one hour at room temperature in the dark with staining reagent (10% of a 5mg/ml lysophosphatidylcholine (Sigma) in 10X HBSS, 80% of the 16% paraformaldehyde, and 10% of 6.6 uM NBD
10 phalloidin (Molecular Probes; Eugene, OR)). After incubation, cells were washed 2X with PBS containing 2% fetal bovine serum (Hyclone, Logan UT) and the fluorescence was quantified on a FACSCAN (Becton Dickinson) gated on activated monocytes. As shown in Figure 13, antibody 4.40 A68G S230P inhibited whole blood actin polymerization in the cynomolgus monkey with an IC₅₀
15 of 0.49 ug/ml.

EXAMPLE 13

Collagen I mRNA quantification assay

[00299] Human hepatic stellate cell line, LI-90 (JCRB Cellbank, Japan), was
20 grown in flasks containing DME medium supplemented with 10% deactivated FBS, 100 U/ml Penicillin/100 ug/ml Streptomycin and 2 mM L-Gln (Invitrogen) at 37°C in a 5% CO₂ humidified incubator. Cells were grown in 96-well tissue culture plates at 20,000 cells/well for 3 days and treated with 1000 nM of MCP-1 (PeproTech) and various concentrations of 4.40 A68G S230P for 48 hours at
25 37°C. The culture medium was removed and the cells were lysed by addition of 100 ul of lysing buffer supplied with QUANTIGENE™ Expression Kit (Panomics). The branched DNA assay was performed following the manufacturer's instructions. Briefly, samples of total RNA were loaded on a 96-well microtiter plate containing hybridization buffer and 50 fmol/ul of Col1A1/GAPDH probe set.
30 The captured mRNAs were hybridized to branched DNA molecules that contained alkaline phosphatase molecules by incubating at 46°C for 60 min. After further incubation with the chemiluminescent substrate at 46°C for 30 min, luminescence was quantified with a luminometer (ARVOsx, Perkin Elmer; Massachusetts, USA).

The ratio of the luminescence of Col1A1 against GAPDH was calculated, and the data was analyzed by Prism 4.0 software to determine IC₅₀ values (GraphPad). As shown in Figure 14, 4.40 A68G S230P inhibited collagen IAI mRNA synthesis in LI90 cells induced by MCP-1 with an IC₅₀ value of 0.89 µg/mL (6.2 nM).

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EXAMPLE 14

pERK phosphorylation

[00300] Human whole blood was freshly isolated from healthy volunteers. FACS analysis indicated a high level of phosphorylation of extracellular signal-regulated kinase (pERK) in CD14+ monocytes 6 min after addition of MCP-1. 4.40 A68G S230P inhibited MCP-1 induced pERK phosphorylation in whole blood in a dose-dependent manner (Figure 15). pERK phosphorylation in monocytes following *ex vivo* stimulation with MCP-1 can be utilized as a mechanism biomarker, thereby facilitating PK/PD and translational pharmacology. The IC₅₀ and IC₉₀ values obtained for the 4.40 A68G S230P antibody in this whole blood assay were 0.44 µg/mL (2.9 nM) and 0.89 µg/ml (6.1 nM), respectively.

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EXAMPLE 15

Murine Acute Hepatitis Model

[00301] Efficacy of the 4.40 A68G S230P antibody was also examined using an acute mouse ConA model of hepatitis. For this study, transgenic mice were used in which mouse CCR2 had been replaced by human CCR2 since the 4.40 A68G S230P antibody does not recognize the rodent CCR2. The animals were given a single intraperitoneal injection of the mAb at 0.1, 0.3 or 1.0 mg/kg in saline. An IgG₄ isotype control antibody was administered to a separate group. Thirty minutes later the animals were given an i.v. tail vein injection (0.1 mL) of 15 mg/kg ConA in pyrogen-free saline. A control group was given saline without ConA. After 24 hours, blood samples were obtained and plasma liver enzymes analyzed. As shown in Figure 16, ALT and AST were markedly elevated in the control antibody group with values close to 25,000 U/L. By contrast, mice treated with 0.5 and 1.0 mg/kg 4.40 A68G S230P showed a significant reduction of their plasma liver enzymes by approximately 50% and 80%, respectively.

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EXAMPLE 17

Therapeutic Effects In Animal Disease Models

Inflammation

- 5 [00302] The therapeutic effects of the human anti-CCR2 antibodies or antigen-binding fragments thereof, including the 4.40 and 4.9 antibodies, are tested using *in vivo* mammalian models of inflammation. Leukocyte infiltration is monitored upon intradermal injection of a chemokine, such as MCP-1, and an antibody or fragment thereof reactive with mammalian CCR2, such as the 4.40 antibody, into
- 10 a suitable animal, such as rabbit, mouse, rat, guinea pig or rhesus macaque (see e.g., Van Damme, J. et al., *J. Exp. Med.* 176:59-65 (1992); Zachariae, C. O. C. et al., *J. Exp. Med.* 171:2177-2182 (1990); Jose, P. J. et al., *J. Exp. Med.* 179: 881-887 (1994)). Skin biopsies are assessed histologically for infiltration of leukocytes (e.g., eosinophils and granulocytes). It is expected that the antagonistic anti-
- 15 CCR2 antibodies will decrease infiltration of leukocyte compared to control animals.

Multiple Sclerosis

- [00303] The therapeutic effects of the antibodies or fragments thereof, including
- 20 the 4.40 and 4.9 antibodies, are tested using *in vivo* mammalian models of multiple sclerosis. Experimental autoimmune encephalomyelitis (EAE) is a T cell-mediated inflammatory disease of the central nervous system (CNS) that serves as an animal model for multiple sclerosis (MS) (see Steinman L, *Neuron* 24:511-514 (1999)). On day 0 and day 7 post-immunization (p.i.) with encephalitogenic
- 25 MOG₃₅₋₅₅ peptide, C57/J129 and/or C57BL/6 mice are sensitized for active EAE by subcutaneous (s.c.) injection (two sites, dorsal flank) with a total of 600 µg of encephalitogenic MOG₃₅₋₅₅ emulsified in incomplete Freund's adjuvant (IFA; Difco, Detroit, MI), containing 70 µg of *Mycobacterium tuberculosis*, H37Ra (Difco). On day 0 and day 2 p.i., each mouse also receives 500 ng of pertussis toxin (PTX;
- 30 List Biological Laboratories, Campbell, CA) intravenously (i.v.) via a tail vein. Some animals also receive an escalated dosage of anti-CCR2 antagonistic antibodies, such as the 4.40 antibody. Animals are assessed daily for clinical signs and evaluated according to the following scale: grade 0, no abnormalities;

grade 1, weak tail; grade 2, limp tail and weakness in hind-limbs; grade 3, hind-limb paraparesis; grade 4, tetraplegia; and grade 5, moribund or death.

[00304] In addition, light microscopy studies are performed on glutaraldehyde/osmium-fixed tissue samples from the optic nerve, cerebrum, cerebellum, and spinal cord. The tissue samples are dehydrated and embedded in epoxy resin from which 1- μ m sections are cut and stained with toluidine blue. Inflammation, demyelination, Wallerian degeneration (WD), and remyelination are scored on a scale of 0 to 5, as described previously (Cannella *et al.*, *Proc Natl Acad Sci USA* 95:10100-10105 (1998)). It is expected that mice treated with anti-CCR2 antagonistic antibodies will exhibit reduced signs of clinical abnormalities, inflammation, demyelination and WD over control animals. It is further expected that such reduction will occur in a dosage-dependent manner.

Neuropathic Pain

[00305] The therapeutic effects of the antibodies or fragments thereof, including the 4.40 and 4.9 antibodies, are tested using *in vivo* mammalian models of neuropathic pain. C57BL mice are divided into two groups. The experimental group is administered anti-CCR2 antagonistic antibodies daily, such as the 4.40 antibody, via tail vein injection while a second group is not administered the antibody. The two groups are tested at time intervals after the initiation of treatment in the following assays:

Rota-rod. Initially, mice are trained on the rota-rod for 3 min at a speed of 10 rpm. For testing, the speed is set at 10 rpm for 60 s and subsequently accelerated to 600 rpm. The time taken for mice to fall after the beginning of the acceleration is recorded.

Hot plate. Mice are habituated to the hot-plate apparatus with temperature set at 45°C for 2 min. Subsequently, mice are placed on the hot-plate and the temperature is sequentially changed to 52.5°C and 55.5°C (cutoff set at 30 s) each and then to 58.5°C (cutoff set at 20 s). The time when mice either lick their paws or jump is recorded.

Formalin test. For 4 days before testing, mice are acclimated for 2 hours every day on the test platform. On the day of the study, mice are placed for 1 hour on the test platform and subsequently are administered 10 μ l of 2% formalin

in the plantar surface of the left paw. The time mice spend either licking or lifting the injected paw is recorded over 2-minute periods at 5-minute intervals for 50 min.

Thermal and mechanical stimulation. Thermal sensitivity is assessed by measuring paw withdrawal latencies to a radiant heat stimulus (Hargreaves *et al.*, *Pain* 32:77-88 (1988)). Mechanical sensitivity is determined with calibrated von Frey filaments by using the up-and-down paradigm (Chaplan *et al.*, *J. Neurosci. Methods* 53, 55-63 (1994)).

Nerve injury. Mice are anesthetized with a mixture of ketamine (50 mg/kg, i.m.) and medetomidine (1 mg/kg, i.m.). An incision is made just below the hip bone, parallel to the sciatic nerve. The nerve is exposed and any adhering tissue is removed from the nerve. A tight ligature with 6-0 silk suture thread around one-third to one-half of the diameter of the sciatic nerve is made. Muscles are closed with suture thread and the wound, with wound clips. The response of the mice to mechanical stimulation is tested before and up to 15 days after nerve injury.

[00306] It is expected that the mice treated with anti-CCR2 antagonistic antibodies will exhibit reduced signs neuropathic pain compared to control mice.

Table 8: CDR Sequences (SEQ ID NO:)

Antibody	V _H CDR1	V _H CDR2	V _H CDR3	V _L CDR1	V _L CDR2	V _L CDR3
4.9	12	13	14	30	31	32
4.22	48	49	50	66	67	68
4.40	84	85	86	102	103	104
4.39	177	178	179	195	196	197

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Claims

1. An isolated human monoclonal antibody or an antigen-binding portion thereof that specifically binds to human CCR2, comprising: a V_H CDR1 as set forth in SEQ ID NO:84, a V_H CDR2 as set forth in SEQ ID NO:85, a V_H CDR3 as set forth in SEQ ID NO:86, a V_L CDR1 as set forth in SEQ ID NO:102, a V_L CDR2 as set forth in SEQ ID NO:103, and a V_L CDR3 as set forth in SEQ ID NO: 104.
2. An isolated human monoclonal antibody or an antigen-binding portion thereof that specifically binds to human CCR2, comprising
 - a heavy chain variable (V_H) domain amino acid sequence comprising SEQ ID NO:83, and
 - a light chain variable (V_L) domain amino acid sequence selected from the group consisting of: SEQ ID NO:101 and SEQ ID NO:113.
3. An isolated human monoclonal antibody or an antigen-binding portion that specifically binds to CCR2, comprising:
 - heavy chain CDR1 , CDR2 and CDR3 of monoclonal antibodies 4.40 produced by hybridoma cells named as ATCC Accession No. PTA-6981 and light chain CDR1, CDR2 and CDR3 of monoclonal antibodies 4.40 produced by hybridoma cells named as ATCC Accession No.PTA-6981.
4. An isolated human monoclonal antibody that specifically binds to human CCR2, comprising
 - a heavy chain having an amino sequence selected from the group consisting of: SEQ ID NO:82 and SEQ ID NO:116, and
 - a light chain having an amino sequence selected from the group consisting of: SEQ ID NO:100 and SEQ ID NO:112.
5. The isolated human monoclonal antibody or antigen binding portion according to claim 4, the antibody having the heavy chain and the light chain selected from the group consisting of:
 - a) the heavy chain having an amino acid sequence of SEQ ID NO:116 and the light chain having an amino acid sequence of SEQ ID NO:112, and
 - b) the heavy chain having an amino acid sequence of SEQ ID NO:82 and the light chain having an amino acid sequence of SEQ ID NO: 100.

6. The isolated human monoclonal antibody or antigen-binding portion according to claim 5, wherein the heavy chain amino acid sequence is SEQ ID NO:116 and the light chain amino acid sequence is SEQ ID NO:112.
7. The isolated human monoclonal antibody or antigen-binding portion according to claim 1, further comprising one or more V_H FR1, FR2, FR3 or FR4 of a heavy chain variable (V_H) domain amino acid sequence of SEQ ID NO:83 and/or V_L FR1, FR2, FR3 or FR4 amino acid sequence of an antibody of a light chain variable (V_L) domain amino acid sequence of SEQ ID NO:101 or SEQ ID NO: 113.
8. The isolated human monoclonal antibody or antigen-binding portion according to claim 7, further comprising a heavy chain constant domain.
9. The isolated human monoclonal antibody or antigen-binding portion according to claim 8, further comprising a light chain constant domain.
10. A pharmaceutical composition comprising the antibody or antigen-binding portion according to claim 1 and a pharmaceutically acceptable carrier.
11. An isolated cell line that produces the antibody or antigen-binding portion according to claim 1.
12. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes the antibody or antigen-binding portion according to claim 1.
13. A vector comprising the nucleic acid molecule according to claim 12, wherein the vector optionally comprises an expression control sequence operably linked to the nucleic acid molecule.
14. A pharmaceutical composition for treating, preventing or alleviating the symptoms of a CCR2- mediated disorder in a subject in need thereof, comprising the antibody or antigen-binding portion according to claim 1.
15. The pharmaceutical composition of claim 14, wherein the subject is afflicted with one or more conditions or disorders selected from the group consisting of an inflammatory disorder, an allergic disorder, an autoimmune disorder, a graft rejection

disorder, atherosclerosis, HIV infection, neuropathic pain, stenosis, restenosis, multiple sclerosis, a fibrotic condition, uveitis, corneal infection, and cancer.

16. The pharmaceutical composition of claim 15, wherein said fibrotic condition is liver fibrosis.
17. The pharmaceutical composition of claim 16, wherein said liver fibrosis is the result of hepatitis C virus (HCV) infection, hepatitis B virus (HBV) infection, alcoholic steatohepatitis (ASH), or non-alcoholic steatohepatitis (NASH).