Abstract: The invention provides novel anti-CXCR5 antibodies and methods of using the same.
Anti-CXCR5 antibodies and methods of use

Field of the Invention

The present invention relates to anti-CXCR5 antibodies and methods of using the same. The anti-CXCR5 antibodies can be used for the treatment of rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus, and/or other B-cell-dependent autoimmune diseases.

Background of the Invention

Rheumatoid arthritis (RA) is an autoimmune disease that has crippling potential and also shortens the life expectancy of the affected patient. Pain and disability are caused by chronic destructive inflammation of small and large joints, referred to as rheumatoid synovitis.

It is believed that B-cells play a role by producing antibodies against self antigens (e.g. rheumatoid factor) and functioning as antigen presenting cells. The combination of MabThera®, a B-cell depleting antibody, and steroids showed equivalent efficacy to anti-TNF antibody therapies. In combination with cyclophosphamide and/or methotrexate the efficacy was even higher. The findings were consistent with the concept that RA is critically dependent on B lymphocytes and suggest that B-lymphocyte depletion may be an effective therapy. Due to the risk involved in depleting most B-cells, targeting B-cell function in a more specific manner (e.g. a subgroup of B-cells) will represent a significant improvement.

CXCR5 is a chemokine receptor cloned originally from a B-cell lymphoma. Its expression is restricted to B-cells and follicular homing T-cells. CXCR5 is expressed by all naive B-cells, follicular B-cells and a subset of memory T-cells that has the phenotype CD4+CD45RO+. Therefore CXCR5 positive B-cells represent a specific subgroup of B-cells. CXCR5 is rarely expressed by CD8+ T-cells and not expressed by naive T-cells.

Only one ligand has been described for this receptor, BCA-1 (BLC, CXCL13). BCA-1 is expressed by stromal cells in lymphoid follicles. CXCL13 (SwissProt accession No. 043927, SCYBD_HUMAN) is the ligand for the chemokine receptor CXCR5, and upon receptor binding it induces Ca^{2+} mobilization, which has been shown in CXCR5 transfected cells. After receptor binding and activation
of signaling cascades, such as elevation of intracellular Ca\(^{2+}\), CXCL13 causes migration of the CXCR5 expressing cells.

In RA patients there is formation of ectopic lymphoid aggregates with germinal center-like structures. BCA-1 RNA was detected in all RA patients around these aggregates. Based on the available evidence, the hypothesis is that BCA-1/CXCR5 play an important role in the formation of lymphoid follicles/germinal centers and through this action modulate B-cell activation. The feasibility of obtaining small molecule antagonists against CXCR5 is unknown.

In WO 95/08576 monoclonal antibodies against leukocyte specific G-protein-coupled receptors are reported. Dual variable domain antibodies and methods for production thereof are reported in US 2010/0047239. In US 2010/0105874 bispecific antibodies and methods for production thereof are reported. Humanized anti-CXCR5 antibodies, derivatives thereof and their use are reported in WO 2009/032661.

**Summary of the Invention**

The invention provides anti-CXCR5 antibodies and methods of using the same.

Herein is reported as one aspect an isolated antibody that specifically binds to human CXCR5.

In one embodiment the antibody inhibits the Ca-flux in CHOal6 cells expressing human CXCR5, which have been induced with 50 nM CXCL13, with an IC\(_{50}\) value of 250 ng/ml or less. In a further embodiment the antibody depletes B-cells from the lymphocyte fraction of whole blood. In another embodiment the antibody is a monoclonal antibody. In one embodiment the antibody is a human or humanized antibody. In a further embodiment the antibody is an antibody fragment that binds CXCR5. In also an embodiment the antibody comprises (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 06, and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 02, or (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 09, (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 12, and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 08, or (a) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 19, and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, or (a) HVR-H3
comprising the amino acid sequence of SEQ ID NO: 23, (b) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27, and (c) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22. In still a further embodiment the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 01, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 02, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, or (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 07, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 08, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 09, or (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, or (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23. In a further embodiment the antibody further comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 04, (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 05, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 06, or (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 10, (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 11, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 12, or (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 17, (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 18, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 19, or (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25, (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27. In one embodiment the antibody further comprises a heavy chain variable domain framework wherein the amino acid sequence of FR1 corresponds to amino acid position 1 to 30 of SEQ ID NO: 16 or SEQ ID NO: 24, the amino acid sequence of FR2 corresponds to amino acid position 36 to 49 of SEQ ID NO: 16 or SEQ ID NO: 24, the amino acid sequence of FR3 corresponds to amino acid position 69 to 100 of SEQ ID NO: 16 or SEQ ID NO: 24, and the amino acid sequence of FR4 corresponds to amino acid position 107 to 117 of SEQ ID NO: 16 or SEQ ID NO: 24. In one embodiment the antibody further comprises a light chain variable domain framework wherein the amino acid sequence of FR1 corresponds to amino acid position 1 to 23 of SEQ ID NO: 20 or SEQ ID NO: 28, the amino acid sequence of FR2 corresponds to amino acid position 41 to 55 of SEQ ID NO: 20 or SEQ ID NO: 28, the amino acid sequence
of FR3 corresponds to amino acid position 63 to 94 of SEQ ID NO: 20 or SEQ ID NO: 28, the amino acid sequence of FR4 corresponds to amino acid position 103 to 112 of SEQ ID NO: 20 or SEQ ID NO: 28. In another embodiment the antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 24, (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 28, or (c) a VH sequence as in (a) and a VL sequence as in (b). In also an embodiment the antibody comprises a VH sequence of SEQ ID NO: 16 or SEQ ID NO: 24. In one embodiment the antibody comprises a VL sequence of SEQ ID NO: 20 or SEQ ID NO: 28.

Another aspect as reported herein is an antibody comprising a VH sequence of SEQ ID NO: 16 or SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 20 or SEQ ID NO: 28. In one embodiment the antibody comprises a VH sequence of SEQ ID NO: 16 and a VL sequence of SEQ ID NO: 20. In one embodiment the antibody comprises a VH sequence of SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 28.

In one embodiment the antibody is a full length antibody of human IgG1 subclass or a full length antibody of human IgG3 subclass. In a further embodiment the antibody is an afucosylated antibody. In another embodiment the antibody is binding to CXCR5 and is glycosylated with a sugar chain at amino acid residue asparagine 297, characterized in that the amount of fucose within the sugar chain is 65% or lower. In one embodiment the antibody is binding to CXCR5 and is glycosylated with a sugar chain at amino acid residue asparagine 297, characterized in that the amount of fucose within the sugar chain is between 5% and 65%, in another embodiment the amount is between 20% and 40%. Antibodies comprising such an amount of fucose are further termed afucosylated. In one embodiment the antibody is binding to CXCR5 and is glycosylated with a sugar chain at amino acid residue asparagine 297, characterized in showing high binding affinity to the human Fc gamma receptor III (FcyRIII). In another embodiment the amount of N-glycol neuraminic acid (NGNA) is 1% or less and/or the amount of N-terminal alpha-1,3-galactose is 1% or less within the sugar chain. In a further embodiment the amount of NGNA is 0.5% or less, and in still a further embodiment the amount is 0.1% or less, and in another embodiment the amount is not detectable by an LCMS method. In one embodiment the amount of N-terminal alpha-1,3-galactose within the sugar chain is 0.5% or less, in a further embodiment 0.1% or less, and in still a further embodiment not detectable by an LCMS method.
One aspect as reported herein is an isolated nucleic acid encoding the antibody as reported herein. Also an aspect as reported herein is a host cell comprising the nucleic acid as reported herein.

Also an aspect as reported herein is a method of producing an antibody comprising the step of culturing the host cell as reported herein so that the antibody is produced. In one embodiment the method comprises the steps of

a) cultivating a cell as reported herein, and
b) recovering the antibody from the cell or the cultivation medium and thereby producing an antibody.

Another aspect is a pharmaceutical formulation comprising the antibody as reported herein and optionally a pharmaceutically acceptable carrier.

An aspect as reported herein is the antibody as reported herein for use as a medicament.

Another aspect as reported herein is the antibody as reported herein for use in treating rheumatoid arthritis, or for use in treating multiple sclerosis, or for use in treating systemic lupus erythematosus, or for use in treating a B-cell dependent autoimmune disease, or for use in inhibiting CXCL13 induced Ca-flux, or for use in depleting B-cells, or for use in treating B-cell lymphomas, or for use in treating cancer.

Also an aspect as reported herein is the use of the antibody as reported herein in the manufacture of a medicament. In one embodiment the medicament is for treatment of rheumatoid arthritis. In also an embodiment the medicament is for treatment of multiple sclerosis. In a further embodiment the medicament is for treatment of systemic lupus erythematosus. In another embodiment the medicament is for treatment of a B-cell dependent autoimmune disease. In still a further embodiment the medicament is for inhibiting CXCL13 induced Ca-flux. In one embodiment the medicament is for depleting B-cells. In a further embodiment the medicament is for treating B-cell lymphomas. In another embodiment the medicament is for treating cancer.

A further aspect as reported herein is a method of treating an individual having rheumatoid arthritis comprising administering to the individual an effective amount of the antibody as reported herein. Another aspect as reported herein is a method of treating an individual having multiple sclerosis comprising administering to the
individual an effective amount of the antibody as reported herein. Still another aspect as reported herein is a method of treating an individual having systemic lupus erythematosus comprising administering to the individual an effective amount of the antibody as reported herein. Also an aspect as reported herein is a method of treating an individual having a B-cell dependent autoimmune disease comprising administering to the individual an effective amount of the antibody as reported herein. One aspect as reported herein is a method of inhibiting CXCL13 induced Ca-flux in an individual comprising administering to the individual an effective amount of the antibody as reported herein to inhibit CXCL13 induced Ca-flux. Another aspect as reported herein is a method of depleting B-cells in an individual comprising administering to the individual an effective amount of the antibody as reported herein to deplete B-cells.

**Brief Description of the Figures**

**Figure 1** shows a representative experiment of the concentration dependent binding of purified murine anti-CXCR5 antibody 2C9 to CHOal6 cells expressing human CXCR5 in cell ELISA. Murine anti-CXCR5 antibody 2C9 did not bind to parental CHOal6 cells (see Example 3).

**Figure 2** shows the induction of Ca-flux by CXCL13 in CHOal6 cells recombinantly expressing human CXCR5 (see Example 4).

**Figure 3** shows the inhibition of CXCL13 induced Ca-flux in CHOocl6 cells expressing human CXCR5 by purified murine anti-CXCR5 antibody 2C9 (see Example 4).

**Figure 4** shows that murine anti-CXCR5 antibody 2C9 does not stimulate the Ca-flux in CHOal6 cells expressing human CXCR5 by itself whereas 30 nM CXCL13 stimulates Ca-flux (see Example 4).

**Figure 5** shows the binding of the humanized antibodies. Daudi target cells were incubated with the antibodies and binding was detected using a fluorescently labeled secondary antibody and subsequent analysis via flow cytometry (FACS) (see Example 7).

**Figure 6** shows the inhibition of CXCL13 (50 nM) induced Ca-mobilization by humanized, glycoengineered anti-CXCR5 antibody (see Example 8).

**Figure 7** shows the depleting activity of different humanized, glycoengineered antibodies (see Example 9).
Detailed Description of Embodiments of the Invention

I. Definitions

An "acceptor human framework" denotes a human antibody framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VH acceptor human framework is identical in sequence to the VH human immunoglobulin framework sequence or human consensus framework sequence. In one embodiment the VH human germline sequence is hVH 3-15. In another embodiment the VL human germline sequence is hVK 4-1.

The term "affinity" denotes the sum total of non-covalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be determined by common methods known in the art, including those described herein.

An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs) or complementarity determining regions (CDRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen, i.e. a reduction of the dissociation constant between an antibody binding site and its binding partner (antigen).

The term "amino acid" denotes the group of carboxy oc-amino acids, which directly or in form of a precursor can be encoded by a nucleic acid. The individual amino acids are encoded by nucleic acids consisting of three nucleotides, so called codons or base-triplets. Each amino acid is encoded by at least one codon. This is known as "degeneration of the genetic code". The term "amino acid" as used within this application denotes the naturally occurring carboxy oc-amino acids comprising alanine (three letter code: ala, one letter code: A), arginine (arg, R), asparagine (asn, N), aspartic acid (asp, D), cysteine (cys, C), glutamine (gin, Q), glutamic acid
(glu, E), glycine (gly, G), histidine (his, H), isoleucine (ile, I), leucine (leu, L), lysine (lys, K), methionine (met, M), phenylalanine (phe, F), proline (pro, P), serine (ser, S), threonine (thr, T), tryptophan (trp, W), tyrosine (tyr, Y), and valine (val, V).

The terms "anti-CXCR5 antibody" and "an antibody that specifically binds to CXCR5" refer to antibodies that are capable of binding CXCR5 with an affinity such that the antibody can be used as a diagnostic and/or therapeutic agent in targeting human CXCR5 in a patient.

The term "antibody" herein is used in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity. Naturally occurring antibodies are molecules with varying structures. For example, native IgG antibodies are hetero tetrameric glycoproteins of about 150,000 Daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable domain (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three or four constant domains (CH1, CH2, CH3 and optionally CH4). Similarly, from N- to C-terminus, each light chain has a variable domain (VL), also called a variable light domain or a light chain variable domain, followed by a constant light chain (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

The term "antibody-dependent cellular cytotoxicity (ADCC)" refers to lysis of human target cells by an antibody as reported herein in the presence of effector cells. ADCC is determined in one embodiment by the treatment of a preparation of CXCR5 expressing cells with an antibody as reported herein in the presence of effector cells such as freshly isolated PBMC or purified effector cells from buffy coats, like monocytes or natural killer (NK) cells or a permanently growing NK cell line.

An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂, diabodies, linear antibodies, single-chain antibody
molecules (e.g. scFv), and multispecific antibodies formed from or comprising antibody fragments.

An "antibody that binds to the same epitope" denotes an antibody that blocks the binding of a reference antibody to its antigen in a competition assay by 50 % or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50 % or more. An exemplary competition assay is provided herein.

"Asn 297" and "asparagine 297" according to the invention denote the amino acid residue asparagine located at about position 297 in the Fc region of an antibody (numbering according to Kabat). Based on minor sequence variations of Fc regions of naturally occurring antibodies, Asn 297 can also be located some amino acids (usually not more than ±3 amino acids) upstream or downstream of position 297, i.e. between position 294 and 300.

The "class" of an antibody refers to the type, especially the amino acid sequence, of the constant domains or constant region possessed by the antibody's heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgGi, IgG2, IgG3, IgG4, IgAl, and IgA2. The sum of the heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

The term "complement-dependent cytotoxicity (CDC)" denotes a process initiated by binding of complement factor Clq to the Fc region of most IgG antibody subclasses. Binding of Clq to an antibody is caused by defined protein-protein interactions at the so called complement-binding site. Such binding sites are known in the state of the art. Further, such binding sites are, e.g., characterized by the amino acids L234, L235, D270, N297, E318, K320, K322, P331, and P329 (numbering according to EU index of Kabat). Antibodies of subclass IgGl, IgG2, and IgG3 usually show complement activation including Clq and C3 binding, whereas IgG4 does not activate the complement system and does not bind Clq and/or C3.

The term "CXCR5," as used herein, refers to any native CXCR5 of human origin. The term encompasses "full-length" unprocessed CXCR5 as well as any form of CXCR5 that results from processing in the cell. The term also encompasses
naturally occurring variants of CXCR5, e.g., splice variants or allelic variants. The amino acid sequence of a human CXCR5 is shown in SEQ ID NO: 29.

The term "depleting" denotes the biological activity of depleting B-cells from the lymphocyte fraction of whole blood samples. The depleting can be determined by using an assay comprising incubating the lymphocyte fraction of whole blood samples from healthy donors with the antibody(ies) in question for 20 hours at 37 °C and determining and quantifying the presence of B-cells via the CD19 marker and of T-cells via the CD3 marker in a flow cytometer, whereby the magnitude of B-cell depletion is calculated by the ratio of B-cells to T-cells prior to and after incubation of the lymphocyte fraction with the antibody.

The term "effector functions" denotes those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: Clq binding and complement dependent cytotoxicity (CDC), Fc receptor binding, antibody-dependent cell-mediated cytotoxicity (ADCC), phagocytosis, down regulation of cell surface receptors (e.g. B-cell receptor) and B-cell activation.

An "effective amount" of an agent, e.g., a pharmaceutical formulation, denotes an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result or effect.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from about residue 226 (Cys), or from about residue 230 (Pro), to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine residue (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, National Institutes of Health, Bethesda, MD (1991).

The term "constant region derived from human origin" denotes a constant heavy chain region of a human antibody of the subclass IgGl, IgG2, IgG3, or IgG4 (comprising e.g. the CH1 domain, the hinge region, the CH2 domain, the CH3 domain, and optionally the CH4 domain) and/or a constant light chain κ or λ region

"Framework" or "FR" denotes variable domain residues other than hypervariable region (HVR) residues or complementarity determining region (CDR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR (CDR) and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4 (FR1-CDRH1(L1)-FR2-CDRH2(L2)-FR3-CDRH3(L3)-FR4).

The terms "full length antibody", "intact antibody", and "whole antibody" are used herein interchangeably to denote an antibody having a structure substantially similar to a native human antibody structure or having heavy chains that contain an Fc region as defined herein.

The terms "host cell", "host cell line", and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants", "transformed cells" and "transfected cells", which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.
A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed., NIH Publication 91-3242, Bethesda MD (1991), Vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al., supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., supra.

A "humanized" antibody refers to an antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term "hypervariable region" or "HVR" as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs, whereof three are in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops or from the "complementarity determining regions" (CDRs), being of highest sequence variability and/or involved in antigen recognition. Hypervariable loops occur in one embodiment at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3) of the VL domain and 26-32 (H1), 53-55 (H2), and 96-101 (H3) of the VH domain (Chothia, C. and Lesk, A.M., J. Mol. Biol. 196 (1987) 901-917). CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur in one embodiment at amino acid residues 24-34 (L1), 50-56 (L2), 89-97 (L3) for the VL domain and 31-35B (H1), 50-65 (H2), and 95-102 (H3) of the VH domain (Kabat et al., Sequences of Proteins of Immunological Interest, 5th ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991)). With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues", or "SDRs", which are residues that contact the antigen.
SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur in one embodiment at amino acid residues 31-34 (L1), 50-55 (L2), 89-96 (L3) of the VL domain and 31-35B (H1), 50-58 (H2), and 95-102 (H3) of the VH domain (see e.g. Almagro, J.C. and Fransson, J., Front. Biosci. 13 (2008) 1619-1633). Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., supra.

An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

An "individual" or "subject" is a mammal. Mammals include, but are not limited to primates (e.g., humans and non-human primates such as monkeys). In certain embodiments, the individual or subject is a human.

An "isolated" antibody is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95 % or 99 % purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., size exclusion, or ion exchange, or reverse phase HPLC) methods. For review of methods for assessment of antibody purity, see, e.g., Flatman, S. et al., J. Chromatogr. B 848 (2007) 79-87.

An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. (An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.)

"Isolated nucleic acid encoding an anti-CXCR5 antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof) of an anti-CXCR5 antibody, including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring
mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, single antibody producing cell isolation methods, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2
sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU5 10087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y,$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

The term "the sugar chains show characteristics of N-linked glycans attached to Asn 297 of an antibody recombinantly expressed in a CHO cell" denotes that the
sugar chain at amino acid residue asparagine 297 of the antibody as reported herein has the same structure and saccharide residue sequence except for the fucose residue as those of the same antibody expressed in unmodified CHO cells, e.g. as those reported in WO 2006/103 100.

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

The term "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to an antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of an antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs) (see, e.g., Kindt et al., Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007)). A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively (see, e.g., Portolano, S. et al., J. Immunol. 150 (1993) 880-887; Clackson, T. et al., Nature 352 (1991) 624-628).

The term "vector", as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors".

II. Compositions and Methods

In one aspect, the invention is based, in part, on the development of a humanized monoclonal antibody directed against a human receptor, also blocking the binding
of the natural receptor ligand. This results in inhibition of calcium mobilization in
these cells and thereby prevents the migration of these cells. In addition the
antibody can be a depleting antibody and exert immune effector functions thereby
eliminating receptor expressing subpopulations of B- or T-lymphocytes. In certain
embodiments, antibodies that bind to CXCR5 are provided. In further embodiments
the anti-CXCR5 antibodies prevent CXCL13 binding to the CXCR5 receptor.
Antibodies of the invention are useful, e.g., for the diagnosis or treatment of
inflammatory arthritis or other auto-immune diseases (or possibly B-cell
lymphoma). It has been found that for maintaining the CXCR5 binding and B-cell
depleting biological activity of the parent murine anti-CXCR5 antibody 2C9 in the
humanized anti-CXCR5 antibody the amino acids at amino acid sequence positions
47 and 49 (numbering according to Kabat) have to be maintained, i.e. transferred,
from the murine amino acid sequence.

A. Exemplary Anti-CXCR5 Antibodies

In one aspect, the invention provides isolated antibodies that specifically bind to
human CXCR5.

In one embodiment, an anti-CXCR5 antibody as reported herein has the property of
specifically binding to human CXCR5. In another embodiment, an anti-CXCR5
antibody as reported herein has the property of preventing CXCL13 binding to the
CXCR5 receptor. In a further embodiment, an anti-CXCR5 antibody as reported
herein has the property of depleting CXCR5 receptor positive T- and B-cells. In
still another embodiment, an anti-CXCR5 antibody as reported herein has the property of
inhibiting calcium mobilization in CXCR5 receptor positive cells. In one
embodiment, an anti-CXCR5 antibody as reported herein has the property of
preventing migration of CXCR5 receptor positive cells. In a further embodiment,
an anti-CXCR5 antibody as reported herein has the property of specifically binding
to human CXCR5 in a cell ELISA with an EC_{50} value of about 0.3 nM. In another
embodiment, an anti-CXCR5 antibody as reported herein has the property of
inhibiting CXCL13 induced calcium flux in CHOocl6 cells expressing human
CXCR5 with an IC_{50} value of about 2 nM. In also an embodiment, an anti-CXCR5
antibody as reported herein has the property of recruiting effector cells like NK
cells or macrophages and thereby depletes target cells. In still another embodiment,
an anti-CXCR5 antibody as reported herein has the property of depleting B-cells
from human whole blood samples with an IC_{50} value of about 1 nM.
In one aspect, the invention provides an anti-CXCR5 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 01, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 02, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 04, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 05, and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 06.

In one aspect, the invention provides an anti-CXCR5 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 07, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 08, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 09, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 10, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 11, and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 12.

In one aspect, the invention provides an anti-CXCR5 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 13, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 14, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 15, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 17, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 18, and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 19.

In one aspect, the invention provides an anti-CXCR5 antibody comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 25, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 26, and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 27.

In one aspect, the invention provides an antibody comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 01, 07, 13, or 21, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 02, 08, 14, or 22, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, 09, 15, or 23. In one embodiment, the antibody comprises a HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, 09, 15, or 23. In another embodiment, the antibody
comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, 09, 15, or 23 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 06, 12, 19, or 27. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, 09, 15, or 23, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 06, 12, 19, or 27, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 02, 08, 14, or 22. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 01, 07, 13, or 21, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 02, 08, 14, or 22, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, 09, 15, or 23.

In another aspect, the invention provides an antibody comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 04, 10, 17, or 25, (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 05, 11, 18, or 26, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 06, 12, 19, or 27. In one embodiment, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 04, 10, 17, or 25, (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 05, 11, 18, or 26, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 06, 12, 19, or 27.

In another aspect, an antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 01, 07, 13, or 21, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 02, 08, 14, or 22, and (iii) HVR-H3 comprising an amino acid sequence selected from SEQ ID NO: 03, 09, 15, or 23, and (b) a VL domain comprising at least one, at least two, or all three VL HVR sequences selected from (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 04, 10, 17, or 25, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 05, 11, 18, or 26, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 06, 12, 19, or 27.

In another aspect, the invention provides an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 01, 07, 13, or 21, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 02, 08, 14, or 22, (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, 09, 15, or 23, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 04, 10, 17, or 25, (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 05, 11, 18, or 26, and (f)
HVR-L3 comprising an amino acid sequence selected from SEQ ID NO: 06, 12, 19, or 27.

In one aspect herein is provided an isolated antibody that binds to human CXCR5, characterized in that the heavy chain variable domain comprises

(a) a CDR-H1 of the amino acid sequence of SEQ ID NO: 01, a CDR-H2 of the amino acid sequence of SEQ ID NO: 02, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 03, or
(b) a CDR-H1 of the amino acid sequence of SEQ ID NO: 07, a CDR-H2 of the amino acid sequence of SEQ ID NO: 08, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 09, or
(c) a CDR-H1 of the amino acid sequence of SEQ ID NO: 13, a CDR-H2 of the amino acid sequence of SEQ ID NO: 14, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 15, or
(d) a CDR-H1 of the amino acid sequence of SEQ ID NO: 21, a CDR-H2 of the amino acid sequence of SEQ ID NO: 22, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 23.

In one embodiment the antibody is further characterized in that the light chain variable domain comprises

(a) a CDR-L1 of the amino acid sequence of SEQ ID NO: 04, a CDR-L2 of the amino acid sequence of SEQ ID NO: 05, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 06, or
(b) a CDR-L1 of the amino acid sequence of SEQ ID NO: 10, a CDR-L2 of the amino acid sequence of SEQ ID NO: 11, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 12, or
(c) a CDR-L1 of the amino acid sequence of SEQ ID NO: 17, a CDR-L2 of the amino acid sequence of SEQ ID NO: 18, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 19, or
(d) a CDR-L1 of the amino acid sequence of SEQ ID NO: 25, a CDR-L2 of the amino acid sequence of SEQ ID NO: 26, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 27.

In one embodiment the antibody is further characterized in that

(a) the heavy chain variable domain comprises a CDR-H1 of the amino acid sequence of SEQ ID NO: 01, a CDR-H2 of the amino acid sequence of SEQ ID NO: 02, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 03, and that that the light chain variable domain comprises a CDR-
L1 of the amino acid sequence of SEQ ID NO: 04, a CDR-L2 of the amino acid sequence of SEQ ID NO: 05, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 06, or
(b) the heavy chain variable domain comprises a CDR-H1 of the amino acid sequence of SEQ ID NO: 07, a CDR-H2 of the amino acid sequence of SEQ ID NO: 08, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 09, and that that the light chain variable domain comprises a CDR-L1 of the amino acid sequence of SEQ ID NO: 10, a CDR-L2 of the amino acid sequence of SEQ ID NO: 11, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 12, or
(c) the heavy chain variable domain comprises a CDR-H1 of the amino acid sequence of SEQ ID NO: 13, a CDR-H2 of the amino acid sequence of SEQ ID NO: 14, and a HVR-H3 of the amino acid sequence of SEQ ID NO: 15, and that that the light chain variable domain comprises a CDR-L1 of the amino acid sequence of SEQ ID NO: 17, a CDR-L2 of the amino acid sequence of SEQ ID NO: 18, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 19, or
(d) the heavy chain variable domain comprises a CDR-H1 of the amino acid sequence of SEQ ID NO: 21, a CDR-H2 of the amino acid sequence of SEQ ID NO: 22, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 23, and that that the light chain variable domain comprises a CDR-L1 of the amino acid sequence of SEQ ID NO: 25, a CDR-L2 of the amino acid sequence of SEQ ID NO: 26, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 27.

In one embodiment the antibody is further characterized in that in the variable heavy chain domain the amino acid at position 47 is arginine and the amino acid at position 49 is alanine.

In one embodiment the antibody is further characterized in that the antibody is a monoclonal antibody.

In one embodiment the antibody is further characterized in that the antibody is a human or humanized antibody.

In one embodiment the antibody is further characterized in that the antibody is an antibody fragment that binds CXCR5.

In one embodiment the antibody is further characterized in comprising
(a) a heavy chain variable domain of an amino acid sequence having at least 95 % sequence identity to the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 24;
(b) a light chain variable domain of an amino acid sequence having at least 95 % sequence identity to the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 28; or
(c) a heavy chain variable domain of an amino acid sequence as in (a) and a light chain variable domain of an amino acid sequence as in (b).

In one embodiment the antibody is further characterized in comprising a heavy chain variable domain of an amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 24.

In one embodiment the antibody is further characterized in comprising a light chain variable domain of an amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 28.

Another aspect as reported herein is an antibody comprising
(a) a heavy chain variable domain of an amino acid sequence of SEQ ID NO: 16 and a light chain variable domain of an amino acid sequence of SEQ ID NO: 20, or
(b) a heavy chain variable domain of an amino acid sequence of SEQ ID NO: 24 and a light chain variable domain of an amino acid sequence of SEQ ID NO: 28.

In one embodiment of the previous aspects the antibody is further characterized in that the antibody is a full length IgGl antibody.

In one embodiment of the previous aspects the antibody is further characterized in that the antibody has a fucose content of more than 0 % to less than 40 %.

In any of the above embodiments, an anti-CXCR5 antibody is a humanized anti-CXCR5 antibody. In one embodiment, an anti-CXCR5 antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, e.g. a human immunoglobulin framework or a human consensus framework. In another embodiment, an anti-CXCR5 antibody comprises HVRs as in any of the above embodiments, and further comprises a heavy chain variable domain framework wherein the amino acid sequence of FR1 corresponds to position 1 to 30 and the amino acid sequence of FR2 corresponds to position 36 to 49 and the amino acid sequence of FR3 corresponds to position 69 to 100 and the
amino acid sequence of FR4 corresponds to position 107 to 117 of SEQ ID NO: 16 or SEQ ID NO: 24. In another embodiment, an anti-CXCR5 antibody comprises HVRs as in any of the above embodiments, and further comprises a light chain variable domain framework wherein the amino acid sequence of FR1 corresponds to position 1 to 23 and the amino acid sequence of FR2 corresponds to position 41 to 55 and the amino acid sequence of FR3 corresponds to position 63 to 94 and the amino acid sequence of FR4 corresponds to position 103 to 112 of SEQ ID NO: 20 or SEQ ID NO: 28.

In another aspect, an anti-CXCR5 antibody comprises a heavy chain variable domain (VH) sequence having at least 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 %, or 100 % sequence identity to the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 24. In certain embodiments, a VH sequence having at least 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, or 99 % identity contains substitutions (e.g. conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-CXCR5 antibody comprising that sequence retains the ability to bind to CXCR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 16 or SEQ ID NO: 24. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-CXCR5 antibody comprises the VH sequence of SEQ ID NO: 16 or SEQ ID NO: 24, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two, or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 01, 07, 13, or 21, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 02, 08, 14, or 22, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, 09, 15, or 23. Also optionally, the anti-CXCR5 antibody comprises the VH sequence of SEQ ID NO: 16 or SEQ ID NO: 24, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two, or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 01, 07, 13, or 21, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 02, 08, 14, or 22, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 03, 09, 15, or 23.

In another aspect, an anti-CXCR5 antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 %, or 100 % sequence identity to the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 28. In certain embodiments, a VL
sequence having at least 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, or 99 % identity contains substitutions (e.g. conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-CXCR5 antibody comprising that sequence retains the ability to bind to CXCR5. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 20 or SEQ ID NO: 28. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-CXCR5 antibody comprises the VL sequence in SEQ ID NO: 20 or SEQ ID NO: 28, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 04, 10, 17, or 25, (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 05, 11, 18, or 26, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 06, 12, 19, or 27. In another particular embodiment, the VL comprises one, two, or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 04, 10, 17, or 25, (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 05, 11, 18, or 26, and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 06, 12, 19, or 27.

In another aspect, an anti-CXCR5 antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In one embodiment, the antibody comprises the VH and VL sequences of SEQ ID NO: 16 and SEQ ID NO: 20, or SEQ ID NO: 24 and SEQ ID NO: 28, respectively, including post-translational modifications of those sequences.

In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-CXCR5 antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-CXCR5 antibody comprising a VH sequence of SEQ ID NO: 16 and a VL sequence of SEQ ID NO: 20, or as an anti-CXCR5 antibody comprising a VH sequence of SEQ ID NO: 24 and a VL sequence of SEQ ID NO: 28.

In a further aspect of the invention, an anti-CXCR5 antibody according to any of the above embodiments is a monoclonal antibody, including a humanized or human antibody. In one embodiment, an anti-CXCR5 antibody is an antibody fragment, e.g., a Fv, Fab, Fab', scFv, diabody, or F(ab')2 fragment. In another embodiment,
the antibody is a full length antibody, e.g., an intact IgG1 antibody or other antibody class or isotype as defined herein.

In a further aspect, an anti-CXCR5 antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below.

1. Antibody Affinity

In certain embodiments, an antibody provided herein has a dissociation constant ($K_D$) of $\leq 10 \text{nM}$, $\leq 1 \text{nM}$, $\leq 0.1 \text{nM}$, $\leq 0.01 \text{nM}$, or $\leq 0.001 \text{nM}$ (e.g. $10^{-8} \text{M}$ or less, e.g. from $10^{-8} \text{M}$ to $10^{-13} \text{M}$, or e.g. from $10^{-9} \text{M}$ to $10^{-13} \text{M}$).

The $K_D$ value can be determined by a surface plasmon resonance method. Binding of an anti-CXCR5 antibody to CXCR5 can be investigated by a BIAcore assay (Pharmacia Biosensor AB, Uppsala, Sweden). The affinity of the binding is defined by the terms $k_a$ (rate constant for the association of the antibody from the antibody/antigen complex), $k_d$ (dissociation constant), and $K_D$ ($k_d/k_a$).

A BIAcore 3000 optical biosensor, CM4 sensor chips, and an amine-coupling kit as obtainable from BIAcore (Uppsala, Sweden) can be used. The anti-CXCR5 antibody or a Fab fragment thereof can be immobilized on a CM4 sensor chip using standard amine-coupling chemistry. HEPES-buffered saline (HBS-P) buffer (10 mM HEPES, 0.15 M NaCl, 0.005 % P20 (Tween® 20), pH 7.4) can be used as the running buffer. The carboxymethyl dextran surface can be activated with a 7-min. injection of a 1:1 ratio of 0.4 M EDC (1-ethyl-3-(3-dimethylaninopropyl) carbodiimide hydrochloride) and 0.1 M NHS (N-hydroxsuccinimide). The anti-CXCR5 antibody can be coupled to the surface with a 7-min. injection of the antibody or antibody fragment in 10 mM sodium acetate (pH 5.5). Remaining activated groups on the chip surface can be blocked with a 7-min. injection of 1 M ethanolamine (pH 8.5).

CXCR5 expressing cells can be solubilized in a buffer consisting of 20 mM Tris (pH 7.0), 0.1 M (NH$_4$)$_2$SO$_4$, 10 % glycerol, 1 protease inhibitor tablet (Roche Diagnostics Indianapolis, IN, USA) per 50 ml buffer, and approximately 1 % lipid/detergent mixture. Of from 1 ml to 2 ml of solubilization buffer can be added to approximately $2 \times 10^6$ cells, sonicated using a probe sonicator (6 x 1 sec. pulses) and placed on a rocker at 4 °C. After 15-120 min. the mixtures can be removed from the rocker and centrifuged at 4 °C for 10 min. at 14,000 rpm using a tabletop
centrifuge. The supernatants containing the CXCR5 can be transferred to new tubes for biosensor analysis.

For BIACore analysis 150 µl of the solubilized receptor can be injected over the surfaces with the immobilized anti-CXCR5 antibody or antibody fragment at a flow rate of 20 µl/min. After the sample loop is washed with running buffer, analyte can be injected to test receptor activity. A water bath attached to the BIACore 3000 can be used to keep the sample blocks at a temperature of 5 °C, and the flow cells within the instrument can be kept at 25 °C for all binding studies. The running buffer can contain 50 mM HEPES (pH 7.5), 150 mM NaCl, 5 mM MgCl₂, 1 mM CaCl₂, 2 mg/ml bovine serum albumin (BSA), and different combinations of detergent and lipid. Captured receptor can be removed from the immobilized anti-CXCR5 antibody using two 10 sec. pulses of regeneration solution (10 mM NaOH comprising 1% n-octyl-β-D glucopyranoside) injected at 100 µl/min.

Association rates (kₐ) and dissociation rates (kₐ) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kₐ) is calculated as the ratio kₐ/kₐ (see, e.g., Chen et al., J. Mol. Biol. 293 (1999) 865-881). If the on-rate exceeds 10⁶ M⁻¹ s⁻¹ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm, emission = 340 nm, 16 nm band-pass) at 25 °C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

2. Antibody Fragments

In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')₂, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson, P.J. et al., Nat. Med. 9 (2003) 129-134. For a review of scFv fragments, see, e.g., Plueckthun, In: The Pharmacology of Monoclonal Antibodies, Vol. 113, Rosenberg and Moore (eds.), Springer-Verlag, New York, pp. 269-315 (1994); WO 93/16185; US 5,571,894 and 5,587,458. For
discussion of Fab and F(\(ab')_2\) fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see US 5,869,046.


Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., US 6,248,516).

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. E. coli or phage), as described herein.

3. Humanized Antibodies

In certain embodiments, the antibody is a "class switched" antibody in which the class or subclass has been changed compared to that of the parent antibody.

In certain embodiments, the antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g. CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.


4. Human Antibodies


Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, N., Nat. Biotech. 23 (2005) 1117-1125. See also, e.g., US 6,075, 181 and US 6,150,584 reporting XENOMOUSE™ technology; US 5,770,429 reporting HuMAB® technology; US 7,041,870 reporting K-M MOUSE® technology; and US 2007/006 1900 reporting VELOCIMOUSE® technology. Human variable regions from intact antibodies generated by such
animals may be further modified, e.g., by combining with a different human constant region.


Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter, G. et al., Ann. Rev. Immunol. 12 (1994) 433-455. Phages typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (e.g., from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths, A.D. et al., EMBO J. 12 (1993) 725-734. Finally, naive libraries can also be made synthetically by cloning non-rearranged V-gene segments from stem cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as reported by Hoogenboom, H.R. and Winter, G. J. Mol. Biol. 227 (1992) 381-388. Patent publications reporting human antibody phage libraries include, for example, US 5,750,373, US 2005/0079574, US 2005/019455, US 2005/0266000, US 2007/017126, US 2007/0160598, US 2007/0237764, US 2007/0292936, and US 2009/0002360.

Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. Multispecific Antibodies

In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for CXCR5 and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of CXCR5. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express CXCR5. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein, C. and Cuello, A.C., Nature 305 (1983) 537-540); WO 93/08829; and Traunecker, A. et al., EMBO J. 10 (1991) 3655-3659), and "knob-in-hole" engineering (see, e.g., US 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for

Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576).

The antibody or fragment herein also includes a "Dual Acting Fab" or "DAF" comprising an antigen binding site that binds to CXCR5 as well as another, different antigen (see, US 2008/0069820, for example).


7. Antibody Variants

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.
a) **Substitution, Insertion, and Deletion Variants**

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions". More substantial changes are provided in Table 1 under the heading of "exemplary substitutions", and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

**Table 1**

<table>
<thead>
<tr>
<th>Original Residue</th>
<th>Exemplary Substitutions</th>
<th>Preferred Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>Val; Leu; Ile</td>
<td>Val</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>Lys; Gin; Asn</td>
<td>Lys</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>Gin; His; Asp, Lys; Arg</td>
<td>Gin</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>Glu; Asn</td>
<td>Glu</td>
</tr>
<tr>
<td>Cys (C)</td>
<td>Ser; Ala</td>
<td>Ser</td>
</tr>
<tr>
<td>Gin (Q)</td>
<td>Asn; Glu</td>
<td>Asn</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>Asp; Gin</td>
<td>Asp</td>
</tr>
<tr>
<td>Gly (G)</td>
<td>Ala</td>
<td>Ala</td>
</tr>
<tr>
<td>His (H)</td>
<td>Asn; Gin; Lys; Arg</td>
<td>Arg</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>Leu; Val; Met; Ala; Phe; Norleucine</td>
<td>Leu</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>Norleucine; He; Val; Met; Ala; Phe</td>
<td>He</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>Arg; Gin; Asn</td>
<td>Arg</td>
</tr>
<tr>
<td>Met (M)</td>
<td>Leu; Phe; He</td>
<td>Leu</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>Trp; Leu; Val; He; Ala; Tyr</td>
<td>Tyr</td>
</tr>
<tr>
<td>Pro (P)</td>
<td>Ala</td>
<td>Ala</td>
</tr>
<tr>
<td>Ser (S)</td>
<td>Thr</td>
<td>Thr</td>
</tr>
<tr>
<td>Thr (T)</td>
<td>Val; Ser</td>
<td>Ser</td>
</tr>
<tr>
<td>Trp (W)</td>
<td>Tyr; Phe</td>
<td>Tyr</td>
</tr>
<tr>
<td>Tyr (Y)</td>
<td>Trp; Phe; Thr; Ser</td>
<td>Phe</td>
</tr>
<tr>
<td>Val (V)</td>
<td>He; Leu; Met; Phe; Ala; Norleucine</td>
<td>Leu</td>
</tr>
</tbody>
</table>
Amino acids may be grouped according to common side-chain properties:

1. hydrophobic: Norleucine, Met, Ala, Val, Leu, lle;
2. neutral hydrophilic: Cys, Ser, Thr, Asn, Gin;
3. acidic: Asp, Glu;
4. basic: His, Lys, Arg;
5. residues that influence chain orientation: Gly, Pro;
6. aromatic: Tip, Tyr, Phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g. improvements) in certain biological properties (e.g. increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

Alterations (e.g. substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots", i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, P.S., Methods Mol. Biol. 207 (2003) 179-196), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been reported, e.g., in Hoogenboom, H.R. et al. in Methods in Molecular Biology 178 (2002) 1-37 (O'Brien et al. (eds..) Human Press, Totowa, NJ). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may
be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind its antigen. For example, conservative alterations (e.g. conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham, B.C. and Wells, J.A., Science 244 (1989) 1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertion variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering
the amino acid sequence such that one or more glycosylation sites is created or removed.

Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn 297 of the CH2 domain of the Fc region (see, e.g., Wright, A. and Morrison, S.L., TIBTECH 15 (1997) 26-32). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid (NANA, Neu5Ac), as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 0 % to 80 %, from 0 % to 65 %, from 0 % to 40 % or from 0 % to 25 %. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn 297, relative to the sum of all glycostructures attached to Asn 297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as reported in WO 2008/077546, for example. Asn 297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues). However, Asn 297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function (see, e.g., US 2003/0157108 and US 2004/0093621). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/01 15614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/01 10704; US 2004/01 10282; US 2004/0109865; WO 2003/0851 19; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO 2005/053742; WO 2002/031 140; Okazaki, A. et al., J. Mol. Biol. 336 (2004) 1239-1249; Yamane-Ohnuki, N. et al., Biotech. Bioeng. 87 (2004) 614-622. Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka, J. et al., Arch. Biochem. Biophys. 249 (1986) 533-545; US 2003/0157108; WO 2004/056312, especially Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-
Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/01 1878; US 6,602,684; and US 2005/0123546. Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.


Cell-mediated effector functions of monoclonal antibodies can be enhanced by engineering their oligosaccharide component as described in Umana, P., et al., Nature Biotechnol. 17 (1999) 176-180, and US 6,602,684. IgG1 type antibodies, the most commonly used therapeutic antibodies, are glycoproteins that have a conserved N-linked glycosylation site at Asn 297 in each CH2 domain. The two complex biantennary oligosaccharides attached to Asn 297 are buried between the CH2 domains, forming extensive contacts with the polypeptide backbone, and their presence is essential for the antibody to mediate effector functions such as antibody dependent cellular cytotoxicity (ADCC) (Lifely, M.R., et al., Glycobiology 5 (1995) 813-822; Jefferis, R., et al., Immunol. Rev. 163 (1998) 59-76; Wright, A.


Therefore in one embodiment of the current invention the monospecific bivalent parent antibody is glycosylated with a sugar chain at Asn 297 whereby the amount of fucose within said sugar chain is 65 % or lower (Numbering according to Kabat). In another embodiment is the amount of fucose within the sugar chain is between 0 % and 65 %, in another embodiment between 20 % and 40 %. In one embodiment the antibody according to the invention is of human IgG1 or IgG3 subclass. In a further embodiment the amount of N-glycolyneuraminic acid (NGNA) is 1 % or less and/or the amount of N-terminal alpha-1,3-galactose is 1 % or less within the sugar chain. The sugar chain show in one embodiment the characteristics of N-linked glycans attached to Asn 297 of an antibody recombinantly expressed in a CHO cell.

c) **Fc region variants**

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g. a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.
In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody in vivo is important but certain effector functions (such as complement activation and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcyR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcRIII only, whereas monocytes express FcyRI, FcRRII and FcRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch, J.V. and Kinet, J.P. (Annu. Rev. Immunol. 9 (1991) 457-492). Non-limiting examples of in vitro assays to assess ADCC activity of a molecule of interest is reported in US 5,500,362 (see, e.g., Hellstrom, L., et al., Proc. Natl. Acad. Sci. USA 83 (1986) 7059-7063 and Hellstrom, L., et al., Proc. Natl. Acad. Sci. USA 82 (1985) 1499-1502; US 5,821,337; Bruggemann, M., et al., J. Exp. Med. 166 (1987) 1351-1361). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTITM non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA, USA), and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI, USA). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in animal model such as that disclosed in Clynes, R. et al., Proc. Natl. Acad. Sci. USA 95 (1998) 652-656. Clq binding assays may also be carried out to confirm that the antibody is unable to bind Clq and hence lacks CDC activity (see, e.g., Clq and C3c binding ELISA inWO 2006/029879 and WO 2005/100402). To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro, H., et al., J. Immunol. Methods 202 (1997) 163-171; Cragg, M.S., et al., Blood 101 (2003) 1045-1052; and Cragg, M.S. and Glennie, M.J., Blood 103 (2004) 2738-2743). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B., et al., Int. Immunol. 18 (2006) 1759-1769).

Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327, and 329 (see, e.g., US 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297, and 327, including the so-called
"DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US 7,332,581).

Certain antibody variants with improved or diminished binding to FcRs are reported (see, e.g., US 6,737,056; WO 2004/056312; Shields, R.L., et al., J. Biol. Chem. 276 (2001) 6591-6604).

In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

In some embodiments, alterations are made in the Fc region that result in altered (i.e. either improved or diminished) Clq binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as reported in US 6,194,551, WO 99/51642, and Idusogie et al., J. Immunol. 164 (2000) 4178-4184.

Antibodies with increased half-lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer, R.L., et al., J. Immunol. 117 (1976) 587-593; Kim, J.K., et al., J. Immunol. 24 (1994) 2429-2434) are reported in US 2005/0014934. Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424, or 434, e.g., substitution of Fc region residue 434 (US 7,371,826).


d) **Cysteine engineered antibody variants**

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118
(EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as reported, e.g., in US 7,521,541.

e) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-l,3-dioxolane, poly-l,3,6-trioxane, ethylene/maleic anhydride copolymer, poly (amino acid)s (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, propypropylene oxide/ethylene oxide copolymers, poly (oxyethylated) polyols (e.g. glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

B. Recombinant Methods and Compositions

Antibodies may be produced using recombinant methods and compositions, e.g., as reported in US 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-CXCR5 antibody as reported herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or amino acid sequence comprising the VH of the antibody (e.g. the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g. expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g. has been transformed or transfected with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the
antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic cell, e.g. a Chinese Hamster Ovary (CHO) cell or a Baby Hamster Kidney (BHK) cell or a Human Embryonic Kidney (HEK) cell, or lymphoid cell (e.g. Y0, NSO, Sp2/0 cell). In one embodiment, a method of making an anti-CXCR5 antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

For recombinant production of an anti-CXCR5 antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g. by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells as reported herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria see, e.g., US 5,648,237, US 5,789,199, and US 5,840,523, also see Charlton, Methods in Molecular Biology, Vol. 248 (Lo, B.K.C. (ed.), Humana Press, Totowa, NJ (2003), pp. 245-254, reporting expression of antibody fragments in E. coli. After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized", resulting in the production of an antibody with a partially or fully human glycosylation pattern (see Gerngross, T.U., Nat. Biotech. 22 (2004) 1409-1414; Li, H. et al., Nat. Biotech. 24 (2006) 210-215).

Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have
been identified which may be used in conjunction with insect cells, particularly for transfection of Spodoptera frugiperda cells.

Plant cell cultures can also be utilized as hosts (see, e.g., US 5,959,177, US 6,040,498, US 6,420,548, US 7,125,978, and US 6,417,429 (reporting PLANTIBODIES™ technology for producing antibodies in transgenic plants).

Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CVI line transformed by SV40 (COS-7), human embryonic kidney line (293 or 293 cells as reported, e.g., in Graham, F.L. et al., J. Gen Virol. 36 (1977) 59-74), baby hamster kidney cells (BHK), mouse Sertoli cells (TM4 cells as reported, e.g., in Mather, J.P., Biol. Reprod. 23 (1980) 243-252), monkey kidney cells (CVI), African green monkey kidney cells (VERO-76), human cervical carcinoma cells (HELA), canine kidney cells (MDCK), buffalo rat liver cells (BRL 3A), human lung cells (W138), human liver cells (Hep G2), mouse mammary tumor cells (MMT 060562), TRI cells, as reported, e.g., in Mather, J.P. et al., Annals N.Y. Acad. Sci. 383 (1982) 44-68, MRC 5 cells, and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR CHO cells (Urlaub, G. et al., Proc. Natl. Acad. Sci. USA 77 (1980) 4216-4220), and myeloma cell lines such as Y0, NSO and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki, P. and Wu, A.M., Methods in Molecular Biology 248 (2004) 255-268).

C. Assays

Anti-CXCR5 antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

1. Binding assays and other assays

In one aspect, an antibody of the invention is tested for its antigen binding activity, e.g., by known methods such as cell ELISA, Western blot, etc.

In another aspect, competition assays may be used to identify an antibody that competes with (1) an antibody that has a VH with the amino acid sequence of SEQ ID NO: 16 and a VL with the amino acid sequence of SEQ ID NO: 20, or (2) an antibody that has a VH with the amino acid sequence of SEQ ID NO: 24 and a VL...
with the amino acid sequence of SEQ ID NO: 28 for binding to CXCR5. In certain
embodiments, such a competing antibody binds to the same epitope (e.g. a linear or
a conformational epitope) that is bound by (1) an antibody that has a VH with the
amino acid sequence of SEQ ID NO: 16 and a VL with the amino acid sequence of
SEQ ID NO: 20, or (2) an antibody that has a VH with the amino acid sequence of
SEQ ID NO: 24 and a VL with the amino acid sequence of SEQ ID NO: 28. Detailed
exemplary methods for mapping an epitope to which an antibody binds
are provided in Morris, G.E. (ed.), Epitope Mapping Protocols, In: Methods in
Molecular Biology, Vol. 66, Humana Press, Totowa, NJ (1996)).

In an exemplary competition assay, immobilized CXCR5 or immobilized cells
expressing CXCR5 can be incubated in a solution comprising a first labeled
antibody that binds to CXCR5 (e.g. an antibody that has a VH with the amino acid
sequence of SEQ ID NO: 16 and a VL with the amino acid sequence of SEQ ID
NO: 20, or an antibody that has a VH with the amino acid sequence of SEQ ID
NO: 24 and a VL with the amino acid sequence of SEQ ID NO: 28) and a second
unlabeled antibody that is being tested for its ability to compete with the first
antibody for binding to CXCR5. The second antibody may be present in a cell (e.g.
hybridoma) supernatant. As a control, immobilized CXCR5 is incubated in a
solution comprising the first labeled antibody but not the second unlabeled
antibody. After incubation under conditions permissive for binding of the first
antibody to CXCR5, excess unbound antibody is removed, and the amount of label
associated with immobilized CXCR5 is measured. If the amount of label associated
with immobilized CXCR5 is substantially reduced in the test sample relative to the
control sample, then that indicates that the second antibody is competing with the
first antibody for binding to CXCR5 (see Harlow and Lane, Antibodies: A
Laboratory Manual, Chapter 14, Cold Spring Harbor Laboratory, Cold Spring
Harbor, NY (1988)).

2. Activity assays

In one aspect, assays are provided for identifying anti-CXCR5 antibodies having
biological activity. Biological activity may include, e.g., reducing or blocking the
binding of CXCL13 to the CXCR5, or depleting CXCR5 expressing cells, or
inhibiting calcium mobilization in CXCR5 positive cells, or inhibiting or
preventing migration of CXCR5 positive cells. Antibodies having such biological
activity in vivo and/or in vitro are also provided.
In certain embodiments, an antibody of the invention is tested for such biological activity.

a) **CXCR5 binding**

CHOocl6 cells expressing human CXCR5 are incubated with a 1:1 mixture of medium and hybridoma supernatant for 2 hours at 4 °C. Afterwards the medium is removed and the cells are fixed with glutaraldehyde. Thereafter a detection antibody (e.g. goat anti-mouse IgG (H & L)-HRP conjugate; Bio-RAD Cat.: 170-6516) is added and incubated for 2 hours at room temperature. After washing unbound detection antibody away 3,3',5,5'-tetramethyl benzidine is added and the reaction is stopped after an incubation time of 5 to 10 min. Optical density (OD) is measured at 450 nm (versus 620 nm).

b) **Calcium flux assay**

At first reference values of the calcium flux induced by incubation of CHOocl6 cells expressing human CXCR5 with CXCL13 in a concentration dependent way are determined. The change in Calcium concentration is measured by incubating the cells with FLIPR Calcium 3 Assay Kit (Molecular Devices Cat-No.: R 8091) and determining the color change by FLEX station measurement according to the protocol of the manufacturer.

For antibody evaluation CHOocl6 cells expressing human CXCR5 are detached from normal culture flask seeded in 96 well assay plates and incubated overnight. A dilution series of the antibody to be tested is prepared in cell culture medium in a separate 96 well plate.

The cell culture medium from the CHOocl6 cells expressing human CXCR5 is removed and a 1:1 dilution of hybridoma supernatant in culture medium is added. After incubation for 1 hour at 37 °C Ca flux is induced by the addition CXCL13, the ligand for CXCR5, and the change in Calcium concentration is measured by incubating the cells with FLIPR Calcium 3 Assay Kit (Molecular Devices Cat-No.: R 8091) and determining the color change by FLEX station measurement according to the protocol of the manufacturer.

c) **Depletion of B-cells**

The lymphocyte fraction of whole blood samples from healthy donors is incubated with the antibody for 20 hours at 37 °C. The presence of B-cells is measured in a
flow cytometer via the CD19 marker, T-cells are quantified via the CD3 marker, and the magnitude of B-cell depletion is calculated with the ratio of B-cells to T-cells with and without antibody incubation.

D. **Immunoonjugates**

The invention also provides immunoonjugates comprising an anti-CXCR5 antibody as reported herein conjugated to one or more further agents, such as a cytotoxic agent.

Conjugates of an antibody and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidom ethyl) cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimideate HC1), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta, E.S., et al., Science 238 (1987) 1098-1104. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triamine pentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody (see WO 94/1 1026). The linker may be a "cleavable linker" facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari, R.V., et al., Cancer Res. 52 (1992) 127-131; US 5,208,020) may be used.

The immunoonjugates herein expressly contemplate, but are not limited to such conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS, GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SIAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinlysulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, IL., US).
E. Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the anti-CXCR5 antibodies provided herein is useful for detecting the presence of CXCR5 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as whole blood, serum, synovial fluid, biopsies, hematopoietic cells, and germinal center-like structures.

In one embodiment, an anti-CXCR5 antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of CXCR5 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-CXCR5 antibody as described herein under conditions permissive for binding of the anti-CXCR5 antibody to CXCR5, and detecting whether a complex is formed between the anti-CXCR5 antibody and CXCR5. Such method may be an in vitro or in vivo method. In one embodiment, an anti-CXCR5 antibody is used to select subjects eligible for therapy with an anti-CXCR5 antibody, e.g., where CXCR5 is a biomarker for selection of patients.

Exemplary disorders that may be diagnosed using an antibody of the invention include systemic lupus erythematosus, Sjögren’s syndrome, inflammatory bowel disease, diabetes, multiple sclerosis, or lymphomas.

In certain embodiments, labeled anti-CXCR5 antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemoluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes $^{32}$P, $^{14}$C, $^{125}$I, $^{3}$H, and $^{131}$I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (US 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or...
microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

F. Pharmaceutical Formulations

Pharmaceutical formulations of an anti-CXCR5 antibody as reported herein are prepared by mixing such antibody having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences - The Science and Practice of Pharmacy 21st edition (2005)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids, antioxidants including ascorbic acid and methionine, preservatives (such as octadeyl dimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol), low molecular weight (less than about 10 residues) polypeptides, proteins, such as serum albumin, gelatin, or immunoglobulins, hydrophilic polymers such as poly (vinylpyrrolidone), amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine, monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins, chelating agents such as EDTA, sugars such as sucrose, mannitol, trehalose or sorbitol, salt-forming counter-ions such as sodium, metal complexes (e.g. Zn-protein complexes), and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rhuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rhuPH20, are reported in US 2005/0260186 and US 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

Exemplary lyophilized antibody formulations are reported in US 6,267,958. Aqueous antibody formulations include those reported in US 6,171,586 and WO 2006/044908, the latter formulations including a histidine-acetate buffer.

The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, especially those with
complementary activities that do not adversely affect each other. For example, it may be desirable to further provide standard of care compounds. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethyl cellulose or gelatin-microcapsules and poly-(methyl methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi permeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules.

The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

G. Therapeutic Methods and Compositions

Any of the anti-CXCR5 antibodies provided herein may be used in therapeutic methods.

In one aspect, an anti-CXCR5 antibody for use as a medicament is provided. In further aspects, an anti-CXCR5 antibody for use in treating rheumatoid arthritis, or multiple sclerosis, or systemic lupus erythematosus, or B cell-dependent autoimmune diseases, or systemic lupus erythematosus, or Sjogren's syndrome, or inflammatory bowel disease, or diabetes, or multiple sclerosis, or lymphomas is provided. In certain embodiments, an anti-CXCR5 antibody for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-CXCR5 antibody for use in a method of treating an individual having rheumatoid arthritis, or multiple sclerosis, or systemic lupus erythematosus, or a B cell-dependent autoimmune diseases, or systemic lupus erythematosus, or Sjogren's syndrome, or inflammatory bowel disease, or diabetes, or multiple sclerosis, or a lymphoma comprising administering to the individual an effective amount of the
antigen anti-CXCR5 antibody as reported herein. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further embodiments, the invention provides an anti-CXCR5 antibody for use in depleting CXCR5 positive B- and/or T-cells, or in inhibiting migration of CXCR5 positive cells. In certain embodiments, the invention provides an anti-CXCR5 antibody for use in a method of depleting CXCR5 positive B- and/or T-cells, or of inhibiting migration of CXCR5 positive cells in an individual comprising administering to the individual an effective amount of the anti-CXCR5 antibody to deplete CXCR5 positive B- and/or T-cells, or to inhibit migration of CXCR5 positive cells. An "individual" according to any of the above embodiments is in one embodiment a human.

In a further aspect, the invention provides for the use of an anti-CXCR5 antibody in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of rheumatoid arthritis, or multiple sclerosis, or systemic lupus erythematosus, or B cell-dependent autoimmune diseases, or systemic lupus erythematosus, or Sjogren's syndrome, or inflammatory bowel disease, or diabetes, or multiple sclerosis, or lymphomas. In a further embodiment, the medicament is for use in a method of treating rheumatoid arthritis, or multiple sclerosis, or systemic lupus erythematosus, or B cell-dependent autoimmune diseases, or systemic lupus erythematosus, or Sjogren's syndrome, or inflammatory bowel disease, or diabetes, or multiple sclerosis, or lymphomas comprising administering to an individual having rheumatoid arthritis, or multiple sclerosis, or systemic lupus erythematosus, or a B cell-dependent autoimmune disease, or systemic lupus erythematosus, or Sjogren's syndrome, or inflammatory bowel disease, or diabetes, or multiple sclerosis, or a lymphoma an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for use in a method of depleting CXCR5 positive B- and/or T-cells or inhibition of migration of CXCR5 positive cells. In a further embodiment, the medicament is for use in a method of depleting CXCR5 positive B- and/or T-cells or inhibition of migration of CXCR5 positive cells in an individual comprising administering to the individual an amount of the medicament effective to deplete CXCR5 positive B- and/or T-cells or to inhibit migration of CXCR5 positive cells. An "individual" according to any of the above embodiments may be a human.
In a further aspect, the invention provides a method for treating rheumatoid arthritis, or multiple sclerosis, or systemic lupus erythematosus, or B cell-dependent autoimmune diseases, or systemic lupus erythematosus, or Sjogren's syndrome, or inflammatory bowel disease, or diabetes, or multiple sclerosis, or lymphomas. In one embodiment, the method comprises administering to an individual having such rheumatoid arthritis, or multiple sclerosis, or systemic lupus erythematosus, or B cell-dependent autoimmune diseases, or systemic lupus erythematosus, or Sjogren's syndrome, or inflammatory bowel disease, or diabetes, or multiple sclerosis, or lymphomas an effective amount of an anti-CXCR5 antibody as reported herein. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human.

In a further aspect, the invention provides a method for depleting CXCR5 positive B- and/or T-cells or inhibiting migration of CXCR5 positive cells in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-CXCR5 antibody to deplete CXCR5 positive B- and/or T-cells or to inhibit migration of CXCR5 positive cells. In one embodiment, an "individual" is a human.

In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-CXCR5 antibodies provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-CXCR5 antibodies provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-CXCR5 antibodies provided herein and at least one additional therapeutic agent, e.g., as described below.

Antibodies of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is a standard of care agent.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies of
the invention can also be used in combination with immune suppressive therapy or chemo therapy or radiation therapy.

An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intraleisional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. Various dosing schedules including but not limited to single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

Antibodies of the invention would be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1% to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

For the prevention or treatment of disease, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1 mg/kg to 10 mg/kg) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage
might range from about 1 μg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody). An initial higher loading dose, followed by one or more lower doses may be administered.

However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-CXCR5 antibody.

H. Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody as reported herein. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody as reported herein, and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a
particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as water for injection (WFI), bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-CXCR5 antibody.

### III. Description of the Sequences

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<th>SEQ ID NO</th>
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IV. EXAMPLES

The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

Example 1
Immunization and generation of hybridomas

Balb/c or NMRI mice were immunized with CHOal6 or murine LI.2 cells recombinantly expressing human CXCR5. The immune response was monitored by testing serum samples of these mice in cell ELISA using CXCR5 transfected CHOal6 versus parental CHOal6 cells. B-cells from mice with sufficient titers of anti-human CXCR5 immunoglobulin were used for immortalization by fusion with mouse myeloma cell line P3X63Ag8.653. Fusions were done according to standard procedures and individual hybridoma supematants screened by cell ELISA for CXCR5 binding antibodies. The relevant hybridomas (supematants binding to CXCR5 expressing cells only) were cloned by single cell FACS sorting two times. A clone from each hybridoma was then cultured in vitro to produce antibody in tissue culture medium for characterization. The antibodies were purified by chromatography on protein G sepharose and then characterized in various biochemical and cellular assays. From this evaluation the hybridoma murine anti-CXCR5 antibody 2C9 has been selected and cloned by FACS sorting.
Example 2
Cell culture and purification of murine anti-CXCR5 antibody 2C9

The hybridoma cell was cultured in medium containing 10% FCS with "ultra-low" bovine IgG content. Purification was achieved by affinity chromatography on protein G Sepharose. The bound material was washed with 1x PBS and eluted with 2.5 mM HCl at pH 2.8. The eluted antibody solution was neutralized and subsequently the buffer was exchanged with 20 mM histidine, 140 mM NaCl, pH 6.0 using an Amicon ultracentrifugation device. General storage temperature was -80 °C after aliquotation. Purified antibody samples were provided for analytical and functional characterization.

Example 3
Cell ELISA

Ten thousand CHOocl6 cells recombinantly expressing human CXCR5 or parental CHOocl6 cells are seeded into each well of a 96 flat well plate and incubated overnight at 37 °C in 100 μl medium.

Thereafter medium is aspirated and 100 μl fresh medium containing purified antibody or a 50 μl : 50 μl mixture of fresh medium and hybridoma supernatant is added and incubated two hours at 4 °C. Medium is aspirated, 100 μl glutaraldehyde (c = 0.05 % in PBS) is added and incubated 10 min. at room temperature. After washing two times with 200 μl PBS, 100 μl detection antibody (e.g. goat anti-mouse IgG (H & L)-HRP conjugate; Bio-RAD Cat.: 170-6516) 1 : 1000 diluted in culture medium is added and incubated for two hours at room temperature. After washing three times with 200 μl PBS, 100 μl 3,3',5,5'-tetramethyl benzidine was added and the reaction was stopped after 5 - 10 min. with 25 μl 1 molal H₂SO₄.

Optical density (OD) is measured at 450 nm (versus 620 nm).

Figure 1 and Table 2 show a representative experiment of the concentration dependent binding of purified murine anti-CXCR5 antibody 2C9 to CHOocl6 cells recombinantly expressing CXCR5 in cell ELISA. Murine anti-CXCR5 antibody 2C9 did not bind to parental CHOocl6 cells.
Example 4

Functional Ca-flux cellular assay

Ca-flux is induced by incubation of CHOocl6 cells recombinantly expressing human CXCR5 with CXCL13 (see Figure 2 and Table 3) in a concentration dependent way. This change in Ca concentration is measured by incubating the cells with FLIPR Calcium 3 Assay Kit (Molecular Devices Cat-No.: R 8091) and measuring the color change by FLEX station measurement (see below) according to the protocol of the manufacturer.

Table 2

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Table 3

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<td>0</td>
<td>1684</td>
</tr>
</tbody>
</table>
FLIPR station Ca-flux assay of CHOal6 cells recombinantly expressing human CXCR5 was used for the measurement of calcium mobilization in response to CXCL13 (BLC) stimulation of CHOal6 huCXCR5 transfected cells.

Cells were detached with EDTA or 0.25 % Trypsin/EDTA and twenty thousand CHOocl6 cells expressing human CXCR5 were seeded per 96 well (black walled, Costar 96 flat well plate, Cat No.: 3904) in 100 µl medium (Culture medium: F-12 (HAM) with 10 % FBS, 1 x Pen/Strep, 1 x Glutamine, 200 µg/ml Hygromycin and 600 µg/ml G418) and incubated overnight. Only the inner 60 wells of the plate were used.

Antibody dilutions were prepared in a different 96 well plate in cell culture medium. The cell culture medium from the wells (black walled Nunc 96 well plate) containing CHOocl6 recombinantly expressing human CXCR5 cells was aspirated (only the inner 60 wells were used). Two hundred microliters medium (containing either purified/control antibody or a 1:1 dilution of hybridoma supernatant in culture medium) was added to each well. For controls pure culture medium was used. Afterwards the wells were incubated at 37 °C for 1 h.

According to the protocol of the manufacturer (FLIPR calcium 3 Assay KIT, Molecular Devices Cat-No.: R8091) 10 ml loading buffer, 10 ml calcium-3 dye, 200 µl fresh 250 mM cone. Probenecid, 400 µl fresh 1 M cone. HEPES buffer were mixed. Thereafter the supernatant from the black walled cell culture plate was removed and 200 µl staining / loading buffer were added. Afterwards the wells were incubated for 1 h at 37 °C. Subsequently the wells were incubated for a further 30 min. at room temperature for temperature equilibration.

During this time prepare plate 2 (Costar 96 well plate) with 150 nM CXCL13 ligand (add 75 µl assay buffer to wells B11, C11, D11 only and to all other 57 wells add 75µl 150 nM (= 5x) CXCL13. Program the FLEX station to add 50 µl from the 75 µl Plate 2 150 nM (= 5x) CXCL13 ligand to plate 1 containing "loaded" cells in 200 µl loading buffer.

Plate 2 has to be prepared with CXCL13 freshly 5-10 min. prior to measuring the calcium flux as the ligand is carrier free and can easily bind to the MTP. CXCL13-CF R&D systems Cat. # 801-CF stock cone. (1 mg/ml = 115 µM) with MW 8.7 kDa diluted 1:766 (5 µl / 3831 µl assay buffer) to obtain 150 nM (= 5x) cone. Solution.
The measure parameters for the FLEX station were as follows: Add 50 µM CXCL13-CF to the cells in 200 µl loading buffer. The compound concentration should be (5x) fold to desired final concentration. Pipette speed: R2 / Time: 240 sec / Interval: 1.6 sec; Excitation wavelength (nm): 485 / Emission wavelength: 525 / Emission cut-off 515 / Graphic of values: Max - Min.

Figure 3 and Table 4 show the inhibition of CXCL13 induced Ca-flux in CHOocl6 cells recombinantly expressing human CXCR5 by purified murine anti-CXCR5 antibody 2C9.

### Table 4

<table>
<thead>
<tr>
<th>sample</th>
<th>antibody concentration [µg/ml]</th>
<th>FLU-FLIRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>6720</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>5646</td>
</tr>
<tr>
<td>3</td>
<td>1.25</td>
<td>6663</td>
</tr>
<tr>
<td>4</td>
<td>0.625</td>
<td>17845</td>
</tr>
<tr>
<td>5</td>
<td>0.313</td>
<td>47939</td>
</tr>
<tr>
<td>6</td>
<td>0.156</td>
<td>52636</td>
</tr>
<tr>
<td>7</td>
<td>0.078</td>
<td>52166</td>
</tr>
<tr>
<td>8</td>
<td>0.039</td>
<td>54269</td>
</tr>
<tr>
<td>9</td>
<td>0.020</td>
<td>57177</td>
</tr>
<tr>
<td>blank</td>
<td>-</td>
<td>3784</td>
</tr>
<tr>
<td>Reference (30 nM CXCL13)</td>
<td>-</td>
<td>52257</td>
</tr>
</tbody>
</table>

### Table 5

<table>
<thead>
<tr>
<th></th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; [ng/ml]</th>
<th>IC&lt;sub&gt;90&lt;/sub&gt; [ng/ml]</th>
</tr>
</thead>
<tbody>
<tr>
<td>murine anti-CXCR5 antibody 2C9</td>
<td>approx. 500</td>
<td>approx. 1000</td>
</tr>
</tbody>
</table>

Figure 4 and Table 6 show that murine anti-CXCR5 antibody 2C9 does not stimulate the Ca-flux in CHOocl6 cells recombinantly expressing human CXCR5 by itself whereas 30 nM CXCL13 stimulates Ca-flux. Therefore murine anti-CXCR5 antibody 2C9 does not act agonistically in this assay.
**Table 6**

<table>
<thead>
<tr>
<th>sample</th>
<th>antibody concentration [µg/ml]</th>
<th>FLU-FLIRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>1470</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>1822</td>
</tr>
<tr>
<td>3</td>
<td>1.25</td>
<td>1680</td>
</tr>
<tr>
<td>4</td>
<td>0.625</td>
<td>1801</td>
</tr>
<tr>
<td>5</td>
<td>0.313</td>
<td>1733</td>
</tr>
<tr>
<td>6</td>
<td>0.156</td>
<td>2036</td>
</tr>
<tr>
<td>7</td>
<td>0.078</td>
<td>1908</td>
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<tr>
<td>8</td>
<td>0.039</td>
<td>1951</td>
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<tr>
<td>9</td>
<td>0.020</td>
<td>2043</td>
</tr>
<tr>
<td>blank</td>
<td>-</td>
<td>1965</td>
</tr>
<tr>
<td>Reference (30 nM CXCL13)</td>
<td>-</td>
<td>40008</td>
</tr>
</tbody>
</table>

**Example 5**

**Cloning and sequence analysis of variable regions of murine anti-CXCR5 antibody 2C9**

Frozen cell pellet from selected hybridoma 2C9 producing antibody against human CXCR5 was thawed. Total RNA was isolated and the synthesis of cDNA was performed. The antibody-specific gene amplification was done using generic 5'-primer and the specific primer shl (5' - GCTCAGAGTGAGGGTCAGACTGC) (SEQ ID NO: 30) for the heavy chain and mk3 (5' - GAAGCTCTTGACAATGGGTGAAGTTG) (SEQ ID NO: 31) for the light chain, respectively.

PCR products were purified using the High Pure PCR product purification kit (Roche Diagnostics GmbH, Mannheim, Germany). PCR products were directly sequenced and cloned into a cloning vector. Isolated plasmid DNA from transformed E.coli cells was re-sequenced to confirm the identity.
Example 6

Humanization of murine anti-CXCR5 antibody 2C9

The murine amino acid sequence was aligned to a collection of human germ-line antibody V-genes, and sorted according to sequence identity and homology. The potential acceptor sequence was selected based on high overall homology and the presence of the right canonical residues already in the acceptor sequence (see Poul, M-A. and Lefranc, M-P., in “Ingenierie des anticorps banques combinares” ed. by Lefranc, M-P. and Lefranc, G., Les Editions INSERM, 1997). The human germ-line sequence IGHV3-15 (IMGT Ac No X92216) was chosen as the acceptor for the heavy chain and sequence IGKV4-1 (IMGT Ac No. Z00023) was chosen for the light chain.

The germ-line V-gene encodes only the region up to the beginning of CDR3 for the heavy chain, and till the middle of CDR3 of the light chain. Therefore, the genes of the germ-line V-genes are not aligned over the whole V-domain. The humanized construct comprised the human frameworks 1 to 3, the murine CDRs and the human framework 4 sequence derived from the human JK4, and the JH4 sequences for light and heavy chain, respectively. The humanized constructs were denoted CXCL for the light chain, and CXCH for the heavy chain.

The genes for those designed antibody sequences were generated by conventional PCR techniques and fused to human IgGl and kappa constant domains for the construction of the expression plasmids.

Antibodies were expressed in mammalian cell culture systems like HEK or CHO, and purified via protein A and size exclusion chromatography.

In the following Table 7 the changes in the different humanized variant chains are listed.

<table>
<thead>
<tr>
<th>antibody chain variant</th>
<th>introduced mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCH1</td>
<td>CDR graft - no additional changes</td>
</tr>
<tr>
<td>CXCH2</td>
<td>S3ON, W47R, S62P, D65G</td>
</tr>
<tr>
<td>CXCH3</td>
<td>S3ON, W47R, Y58D, D61A</td>
</tr>
<tr>
<td>CXCH4</td>
<td>S3ON, W47R</td>
</tr>
<tr>
<td>CXCH5</td>
<td>S3ON</td>
</tr>
</tbody>
</table>
Glycoengineering of the antibodies was performed to enhance the immune effector functions to increase ADCC on target cells. Essentially, two enzymes that occur in the late Golgi apparatus of mammalian cells were genetically modified to be expressed in the early Golgi instead. This results in a modified structure of the naturally occurring sugar in the constant part of the antibody. This leads to increased affinity of the Fc portion towards the Fc gamma receptor III, and subsequent to enhanced recruitment of effector cells like NK cells or macrophages.

**Example 7.**

**Binding of humanized antibodies to human CXCR5 expressing cells**

In Figure 5 binding of the humanized antibodies is shown. Daudi target cells were incubated with the antibody and binding was detected using a fluorescently labeled secondary antibody and subsequent analysis via flow cytometry (FACS).

The different combinations of light and heavy chains showed almost no difference in antigen binding, whereby combinations comprising heavy chain variants H3, H5 and H6 showed reduced antigen binding.

**Example 8.**

**Humanized antibodies inhibit CXCL13 induced Ca-flux**

Humanized and glycoengineered antibody H4BL2 was tested in functional assay involving inhibition of CXCL13 induced calcium mobilization as described in Example 4.

In Figure 6 and Table 8 the inhibition of CXCL13 (50 nM) induced Ca mobilization by the humanized, glycoengineered anti-CXCR5 antibody is shown.
The humanized and glycoengineered MAb potently inhibits CXCL13 (50 nM) induced Ca mobilization (IC\textsubscript{50} about 200 ng/ml).

**Table 8**

<table>
<thead>
<tr>
<th>sample</th>
<th>antibody concentration [ng/ml]</th>
<th>FLU-FLIRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4000</td>
<td>401</td>
</tr>
<tr>
<td>2</td>
<td>2000</td>
<td>428</td>
</tr>
<tr>
<td>3</td>
<td>1000</td>
<td>345</td>
</tr>
<tr>
<td>4</td>
<td>500</td>
<td>713</td>
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<tr>
<td>5</td>
<td>250</td>
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<td>125</td>
<td>10101</td>
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<tr>
<td>7</td>
<td>62.5</td>
<td>10866</td>
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<td>8</td>
<td>31.3</td>
<td>11682</td>
</tr>
<tr>
<td>blank</td>
<td>-</td>
<td>1094</td>
</tr>
<tr>
<td>Reference (50 nM CXCL13)</td>
<td>-</td>
<td>12988</td>
</tr>
</tbody>
</table>

**Example 9**

**Humanized antibodies exert ADCC**

The lymphocyte fraction of whole blood samples from healthy donors was incubated with the indicated antibodies for 20 hours at 37 °C. The presence of B-cells was measured in a flow cytometer via the CD19 marker, T-cells were quantified via the CD3 marker, and the magnitude of B-cell depletion was calculated with the ratio of B-cells to T-cells with and without antibody incubation.

Glycoengineering of the antibodies was performed to enhance the immune effector functions to increase ADCC on target cells. Essentially, two enzymes that occur in the late Golgi apparatus of mammalian cells were genetically modified to be expressed in the early Golgi instead. This results in a modified structure of the naturally occurring sugar in the constant part of the antibody. This leads to increased affinity of the Fc portion towards the Fc gamma receptor III, and subsequent to enhanced recruitment of effector cells like NK cells or macrophages.

The glycoengineering was performed according to the methods reported in WO 99/054342 and WO 04/065540, which are incorporated herein by reference.
In summary in the methods host cells to alter the glycosylation profile of one or more polypeptides produced by that host cell were employed. The methods were used to produce antibodies with modified glycosylation in the Fc region, including reduced fucosylation, so that the antibodies have increased effector function and/or increased Fc receptor binding as a result of the modified glycosylation.

The employed host cells are glycoengineered to express a nucleic acid molecule encoding a polypeptide with GnTIII (B(1,4)-N-acetylglucosaminyltransferase) catalytic activity or GalT catalytic activity and optionally to coexpressed a nucleic acid molecule encoding a polypeptide having human ManII (mannosidase II) catalytic activity and/or a nucleic acid molecule encoding a polypeptide with GnTII (B(1,2)-N-acetylglucosaminyltransferase II) catalytic activity.

As can be seen in Figure 7 and Table 9 the humanized, glycoengineered antibody shows depleting activity.

### Table 9

<table>
<thead>
<tr>
<th>sample</th>
<th>antibody concentration [ng/ml]</th>
<th>B-cell depletion [%]</th>
<th>H1L1</th>
<th>H1L2</th>
<th>H4L1</th>
<th>H4L2</th>
<th>H5L1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0.017</td>
<td>8.3</td>
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<td>-1.7</td>
<td>7.5</td>
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<td></td>
</tr>
<tr>
<td>3</td>
<td>0.12</td>
<td>2.6</td>
<td>5.6</td>
<td>-0.4</td>
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<td>-0.9</td>
<td></td>
</tr>
<tr>
<td>4</td>
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<td></td>
</tr>
<tr>
<td>5</td>
<td>5.8</td>
<td>35.3</td>
<td>33.9</td>
<td>34.5</td>
<td>37.9</td>
<td>-1.9</td>
<td></td>
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<tr>
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<td>41</td>
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<td>47.7</td>
<td>45.3</td>
<td>37.5</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>286</td>
<td>39.0</td>
<td>47.4</td>
<td>46.5</td>
<td>40.1</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2000</td>
<td>38.6</td>
<td>45.0</td>
<td>52.5</td>
<td>38.8</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>EC50 [ng/ml]</td>
<td>≈0.88</td>
<td>2.7</td>
<td>0.99</td>
<td>≈1.04</td>
<td>n.d.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n.d. = not determined

To further increase the biological activity of the humanized, glycoengineered antibody the humanized variant chains listed in Table 10 have been generated.
Table 10

<table>
<thead>
<tr>
<th>antibody chain variant</th>
<th>introduced mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCH4A</td>
<td>W47R, T93V</td>
</tr>
<tr>
<td>CXCH4B</td>
<td>W47R, G49A</td>
</tr>
<tr>
<td>CXCH4C</td>
<td>N35S, W47R</td>
</tr>
<tr>
<td>CXCH4D</td>
<td>S7T, K13Q, G15K, R19K, S30N, W47R</td>
</tr>
</tbody>
</table>

The depleting activity of exemplary variant chains are shown in Table 11.

Table 11

<table>
<thead>
<tr>
<th>sample</th>
<th>antibody concentration [ng/ml]</th>
<th>B-cell depletion [%]</th>
<th>H4L2</th>
<th>H4BL2</th>
<th>H4GL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
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<td>-1.8</td>
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<td>2.1</td>
<td></td>
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<td>11.0</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
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<td>2.0</td>
<td>28.1</td>
<td>27.8</td>
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</tr>
<tr>
<td>6</td>
<td>3.1</td>
<td>9.9</td>
<td>38.9</td>
<td>36.5</td>
<td></td>
</tr>
<tr>
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<td>13</td>
<td>30.7</td>
<td>37.6</td>
<td>44.6</td>
<td></td>
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<tr>
<td>8</td>
<td>50</td>
<td>38.6</td>
<td>40.1</td>
<td>44.0</td>
<td></td>
</tr>
<tr>
<td>EC50 [ng/ml]</td>
<td>2.89</td>
<td>0.34</td>
<td>0.88</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.
Patent Claims

1. An antibody that binds to human CXCR5, characterized in that the heavy chain variable domain comprises

   (a) a CDR-H1 of the amino acid sequence of SEQ ID NO: 01, a CDR-H2 of the amino acid sequence of SEQ ID NO: 02, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 03, or

   (b) a CDR-H1 of the amino acid sequence of SEQ ID NO: 07, a CDR-H2 of the amino acid sequence of SEQ ID NO: 08, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 09, or

   (c) a CDR-H1 of the amino acid sequence of SEQ ID NO: 13, a CDR-H2 of the amino acid sequence of SEQ ID NO: 14, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 15, or

   (d) a CDR-H1 of the amino acid sequence of SEQ ID NO: 21, a CDR-H2 of the amino acid sequence of SEQ ID NO: 22, and a CDR-H3 of the amino acid sequence of SEQ ID NO: 23.

2. The antibody of claim 1, further characterized in that the light chain variable domain comprises

   (a) a CDR-L1 of the amino acid sequence of SEQ ID NO: 04, a CDR-L2 of the amino acid sequence of SEQ ID NO: 05, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 06, or

   (b) a CDR-L1 of the amino acid sequence of SEQ ID NO: 10, a CDR-L2 of the amino acid sequence of SEQ ID NO: 11, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 12, or

   (c) a CDR-L1 of the amino acid sequence of SEQ ID NO: 17, a CDR-L2 of the amino acid sequence of SEQ ID NO: 18, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 19, or

   (d) a CDR-L1 of the amino acid sequence of SEQ ID NO: 25, a CDR-L2 of the amino acid sequence of SEQ ID NO: 26, and a CDR-L3 of the amino acid sequence of SEQ ID NO: 27.
3. The antibody of any one of the preceding claims, wherein in the variable heavy chain domain the amino acid at position 47 is arginine and the amino acid at position 49 is alanine.

4. The antibody of any one of the preceding claims, wherein the antibody is a monoclonal antibody.

5. The antibody of any one of the preceding claims, wherein the antibody is a human or humanized antibody.

6. The antibody of any one of the preceding claims, wherein the antibody is an antibody fragment that binds CXCR5.

7. The antibody of any one of the preceding claims, comprising

   (a) a heavy chain variable domain of an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 24;

   (b) a light chain variable domain of an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 28; or

   (c) a heavy chain variable domain of an amino acid sequence as in (a) and a light chain variable domain of an amino acid sequence as in (b).

8. The antibody of any one of the preceding claims, comprising a heavy chain variable domain of an amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 24.

9. The antibody of any one of the preceding claims, comprising a light chain variable domain of an amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 28.

10. An antibody comprising

    (a) a heavy chain variable domain of an amino acid sequence of SEQ ID NO: 16 and a light chain variable domain of an amino acid sequence of SEQ ID NO: 20, or
(b) a heavy chain variable domain of an amino acid sequence of SEQ ID NO: 24 and a light chain variable domain of an amino acid sequence of SEQ ID NO: 28.

11. The antibody of any one of the preceding claims, which is a full length IgG1 antibody.

12. The antibody of any one of the preceding claims, which has a fucose content of more than 0% to less than 40%.

13. Isolated nucleic acid encoding the antibody of any one of claims 1 to 12.


15. A method of producing an antibody comprising culturing the host cell of claim 14 so that the antibody is produced.

16. The method of claim 15 comprising

   a) providing a cell according to claim 14,
   b) cultivating the cell, and
   c) recovering the antibody from the cell or the cultivation medium and thereby producing an antibody.

17. A pharmaceutical formulation comprising the antibody of any one of claim 1 to 12 and optionally a pharmaceutically acceptable carrier.

18. The antibody of any one of claim 1 to 12 for use as a medicament.

19. The antibody of any one of claim 1 to 12 for use in treating rheumatoid arthritis.

20. The antibody of any one of claim 1 to 12 for use in treating multiple sclerosis.

21. The antibody of any one of claim 1 to 12 for use in treating systemic lupus erythematosus.

22. The antibody of any one of claim 1 to 12 for use in treating a B-cell dependent autoimmune disease.
23. The antibody of any one of claim 1 to 12 for use in treating B-cell lymphomas.

24. The antibody of any one of claim 1 to 12 for use in inhibiting CXCL13 induced Ca-flux.

25. The antibody of any one of claim 1 to 12 for use in depleting B-cells.

26. Use of the antibody of any one of claim 1 to 12 in the manufacture of a medicament.

27. The use of claim 26, wherein the medicament is for treatment of rheumatoid arthritis.

28. The use of claim 26, wherein the medicament is for treatment of multiple sclerosis.

29. The use of claim 26, wherein the medicament is for treatment of systemic lupus erythematosus.

30. The use of claim 26, wherein the medicament is for treatment of a B-cell dependent autoimmune disease.

31. The use of claim 26, wherein the medicament is for treatment of cancer.

32. The use of claim 26, wherein the medicament is for inhibiting CXCL13 induced Ca-flux.

33. The use of claim 26, wherein the medicament is for depleting B-cells.

34. A method of treating an individual having rheumatoid arthritis comprising administering to the individual an effective amount of the antibody of any one of claim 1 to 12.

35. A method of treating an individual having multiple sclerosis comprising administering to the individual an effective amount of the antibody of any one of claim 1 to 12.
36. A method of treating an individual having systemic lupus erythematosus comprising administering to the individual an effective amount of the antibody of any one of claim 1 to 12.

37. A method of treating an individual having a B-cell dependent autoimmune disease comprising administering to the individual an effective amount of the antibody of any one of claim 1 to 12.

38. A method of treating an individual having a tumor of the lymphatic system comprising administering to the individual an effective amount of the antibody of any one of claim 1 to 12.

39. A method of inhibiting CXCL13 induced Ca-flux in an individual comprising administering to the individual an effective amount of the antibody of any one claim 1 to 12 to inhibit CXCL13 induced Ca-flux.

40. A method of depleting B-cells in an individual comprising administering to the individual an effective amount of the antibody of any one claim 1 to 12 to deplete B-cells.
Figure 7

% B cell depletion

Ab conc. (ng/ml)
Figure 7

b)

Graph showing % B cell depletion against ab conc. (ng/ml) with different markers for CXC H4L2 G2(1), CXC H4L2 G2(1), and CXC H4L2 G2(1).
INTERNATIONAL SEARCH REPORT

PCT/EP2011/062320

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/28 A61K39/395
ADD.

According to International Patent Classification (IPC) or both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database consulted during the international search (name of database and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search 5 September 2011

Date of mailing of the international search report 23/09/2011

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

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TABLE 1-1-40

**C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT**

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<td>LISIGNOLI GINA ET AL: &quot;Human osteoblasts express functional CXC chemokine receptors 3 and 5: Activation by their ligands CXCL10 and CXCL13 significantly induces alkaline phosphatase and beta-N-acetyl hexosaminidase release.&quot;, JOURNAL OF CELLULAR PHYSIOLOGY, vol. 194, no. 1, January 2003 (2003-01), pages 71-79, XP002654952, ISSN: 0021-9541 page 73, left-hand column, paragraph 1</td>
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